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(57) Abstract

The present invention relates to an anti-meningococcal vaccine, particularly for group-B serotype meningococcus. The invention provides antigenic peptide ligands which can act as an immunogen capable of eliciting an immune response to produce antibodies against the capsular polysaccharide of group-B meningococci (CPS-B).

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Meningococcal Vaccine

The present invention relates to an anti-meningococcal vaccine, particularly for group-B serotype meningococcus. The invention provides antigenic peptide ligands which can act as an immunogen capable of eliciting an immune response to produce antibodies against the capsular polysaccharide of group-B meningococci (CPS-B). The immunogen may be in the form of a polypeptide or in the form of a conjugate of such a polypeptide coupled to a carrier molecule such as a protein or in the form of a peptide displayed upon a virus particle (ie. a virion), for example a bacteriophage. The immunogen may be used in treatment of or prophylaxis against group-B meningococcal infection. The invention also provides anti-group-B-meningococcal antibodies for use in treatment and/or prophylaxis. In addition, the invention relates to methods for producing such immunogens, antibodies and vaccines and relates to their use in methods of treatment and prophylaxis.

Infection by group-B Meningococci (*Neisseria meningitidis*) is the major cause of bacterial meningitis in the UK, EC and worldwide[1-9]. In the UK approximately 60% of the bacterial meningitis cases are due to group-B meningococci. The majority of cases occur in infants of 3 months to 3 years of age. Despite the best efforts of medical staff, and the rapid use of antibiotic and other intervention strategies the mortality rates in the UK still hover around the 2-6% for meningococcal meningitis and 14-50% for meningococcal septicaemia. There is also a considerable amount of post-infection morbidity in

survivors. There is therefore a requirement for a vaccine to group-B meningococci. Indeed vaccines against other serotypes of meningococci, including groups A, C and Y have been successfully produced[10-12]. These vaccines all utilise capsular polysaccharide material to elicit a protective immune response, but the responses are serogroup specific and to date there has been little success in efforts to produce an effective vaccine to the B-group meningococci, using similar approaches[13]. The current status of attempts to obtain a B-group meningococcal vaccine has been reviewed by Romero et al[14(a)], and the background to vaccines against meningococcal diseases has been reviewed by Ala'aldeen et al[14(b)].

Unlike the capsular polysaccharide of other serogroups, that of the B-group meningococci is very poorly immunogenic. This is probably due to a number of factors, including the sensitivity of the polysaccharide to degradation by host enzymes and also the similarity of the polysaccharide structure to carbohydrate components present within the host tissues[15-17]. The capsular polysaccharide of group-B meningococci is a homopolymer of repeated residues of α-(2-8)-linked oligomers of sialic acid: more specifically it is a linear polymer of α(2'-8) linked '-N-acetylneuraminic acid residues[18-20] (2'-8 'NANA). This particular form of polysaccharide capsule material is found only in group-B meningococci, *E. coli* K1 strain, which is also a human pathogen, *Pastuerella haemolytica*, a sheep pathogen and *Moraxella nonliquefaciens* a non-pathogenic commensal[21]. When isolated from *E. coli* K1 the polysaccharide is referred to as "Colominic acid".

Efforts have to a large extent turned away from the use of capsular polysaccharide alone as a potential vaccine material, and concentrated on other components of bacterium (such as fimbriae, outer membrane proteins (OMPs) etc.), without noticeable success in terms of potential vaccines.

The general consensus of data concerning A, C, Y and W135 serogroup specific meningococcal vaccines already available suggests that capsular polysaccharide material, used as a vaccine, elicits protective immune responses. Indeed immunoglobulins which bind the group-B capsular polysaccharide (both IgM and IgG classes) can be shown to have bactericidal activity in vitro and protect against in vivo challenge with lethal doses of *N. meningitidis* or *E. coli* K1. However, the capsular polysaccharide of group-B meningococci appears unsuitable for effective use as a vaccine material because it is non-immunogenic or very weakly immunogenic even when coupled to carrier proteins.

An extract from the conclusions of the Romero survey [14(a)] reads as follows:

> "The search for an ideal antigen as a potential candidate for an *N. meningitidis* B vaccine has made the outer surface of this organism one of the most widely studied cell coats. Not only have the results advanced our understanding of the different mechanisms of associated virulence and pathogenicity, but the research techniques developed have often been applicable to the development of vaccines that protect against other capsulated bacterial pathogens.

Hence, one of the *H. influenzae* type B vaccines consists of a capsular polysaccharide conjugate to meningococcal outer membrane proteins that are used for their special immunogenic properties.

For an antigen to be considered a vaccine candidate for the entire serogroup B, two fundamental requirements must be fulfilled: it must be highly conserved among the different strains, and it must induce bactericidal antibodies. The latter is of particular importance in protection against *N. meningitidis*. To date, it does not seem that these characteristics can be found in a single candidate vaccine, so a multifaceted approached to the problem is needed in order to meet this goal".

An extract from the conclusions of the Ala'aldeen survey [14(b)] reads as follows:

"It is evident that there has been considerable activity in the meningococcal vaccine field over the past few years and there is now room for some optimism. The advances in the development of polysaccharide conjugate vaccines should eventually lead to much more effective vaccines against Group A and C disease, in particular to vaccines that will be effective in the youngest children who are indeed most at risk. However, the problems of a group-B meningitis vaccine remain."

The current state of the art does not allow the ab-initio design of antigenic ligands, even given an antibody

structure (such as an X-ray crystallographic structure). In the specific case of peptides mimicking the relevant conformational structure of the group-B meningococcal polysaccharide, ab-initio design would be particularly difficult since there is no knowledge available as to what amino acid residues or sequences might be suitable as mimetics of this structure. Although studies by other investigators (Hoess, Scott, Devlin) have identified peptides mimicking monosaccharide or oligosaccharides, these data are not sufficient to make any useful predictions about the residues, sequences or structures of peptides that might bind an anti-group-B polysaccharide antibody.

Conventional approaches to the development of vaccines against infectious disease use the infectious agent in an inactivated or attenuated form, or some part of that agent, as a basis for the vaccine. The approach we have adopted uses instead an antibody which embodies a surface which is complementary to some site on the agent (in terms of shape, charge etc.). According to this method, the antibody is used (metaphorically) as a mould to fashion peptide ligands which represent the corresponding site on the infectious agent. Preferably the site, and the corresponding antibody comprise a neutralising site and a neutralising antibody respectively - such that attachment of the antibody to the infectious agent inactivates the infectious agent or toxic products of that agent.

The process of generating peptides representative of the infectious agent is accomplished using libraries of synthetic or phage-displayed random peptides. Members of

peptide libraries that fit the antibody well comprise candidates for incorporation into vaccines, for example, in the form of peptide/carrier protein conjugates, or in the form of phage-peptide constructs.

In a first aspect the invention provides a pharmaceutical compound which includes a chemical composition capable of adopting a structure essentially equivalent to the pharmacophoric pattern of a section of the capsular polysaccharide of *Neisseria meningitidis* group B, optionally together with a pharmaceutically acceptable carrier or excipient for use as an anti-group B capsular polysaccharide immunogen.

In a second aspect the invention provides peptides which include an amino acid sequence capable of adopting a structure having an pharmacophoric pattern essentially equivalent to the pharmacophoric pattern of a section of the capsular polysaccharide of *Neisseria meningitidis* group B.

In a third aspect the invention provides antigenic peptide ligands which cross react with antibodies against the capsular polysaccharide of group B meningococci (CPS-B) which peptides include 1 or more copies of a motif which consists of a ring aromatic moiety, a spacer moiety, and a moiety having negative charge. In addition to these 3 key moieties there is a further optional moiety which is a possible additional hydrogen bond donor/acceptor. Such peptides may have two or more of said motifs which may repeat sequentially or may repeat in an overlapping frame.

In a fourth aspect the invention provides an antigenic peptide which includes an amino acid sequence and structure essentially equivalent to the pharmacophore defined as follows:

the pharmacophore includes at least 3 chemical features; a Ring Aromatic, a Negative Charge and a Hydrogen Bond Acceptor or Donor feature. In addition to these 3 key chemical features there is a further optional feature which is a possible additional hydrogen bond donor/acceptor. The key features are further defined as follows:

(1A) the hydrogen bond acceptor feature matches the following atom types or groups of atoms which are surface accessible;

- sp or sp^2 nitrogens that have a lone pair and a charge less than or equal to zero
- sp^3 oxygens or sulphurs that have a lone pair and charge less than or equal to zero
- non-basic amines that have a lone pair;

(1B) the hydrogen bond donor feature has the same chemical characteristics as the hydrogen bond acceptor except that it also includes basic nitrogen (there is no exclusion of electron-deficient pyridines and imidazoles);

this feature matches the following atom types or groups of atoms;

- non-acidic hydroxyls
- thiols
- acetylenic hydrogens
- NH moieties (except tetrazoles and trifluoromethyl sulfonamide hydrogens);

(2) the negative charge feature is defined as a negative charge not adjacent to a positive charge; and

(3) the ring aromatic feature is defined as an aromatic moiety which may be replaced by amino acid residues having hydrophobic character such as the amino acid residues selected from the following group;

methionine, alanine, leucine, isoleucine, valine, proline; and

(ia) the hydrogen bond acceptor is represented by a vector function consisting of two spheres;

the smaller sphere (at least 1.6Angstroms radius up to 2.6 Angstroms) defines the centroid of the hydrogen bond acceptor on the ligand while the large sphere (at least 2.2Angstroms radius up to 2.6 Angstroms) defines the projected point of the hydrogen bond acceptor from the receptor;

these two spheres are at least 3.0Angstroms apart;

(ib) the hydrogen bond donor is represented by a two sphere vector function as (ia) above;

(ii) the negative charge is represented by a sphere at least 1.6Angstroms radius (up to 2.6 Angstroms); and

(iii) the ring aromatic is represented as two equal size spheres (at least 1.6Angstroms radius up to 2.0 Angstroms) whose centroids are 3.1Angstroms apart;

one sphere corresponds to the position of an aromatic ring moiety and the other to the projected point of the electron pi stacking of the aromatic ring system;

and wherein the tolerances on all distances between these features is +/- 0.5 Angstroms and the geometric angles +/- 20 Degrees and said distances and angles are shown in figure 1 or figure 2.

In a fifth aspect the invention provides antigenic peptides of 3 to 25 amino acids of the general formula:

X-B-C-D-Y

wherein

X is any group of amino acids of length P;

D is any hydrophobic amino acid group;

C is any amino acid;

B is any negative residue;

Y is any group of amino acids of length Q; and

P and Q may be zero and P + Q is less than or equal to 22.

Note that since X and Y may be absent or identical the above defined "motif" may be read in either direction in many circumstances.

These peptides preferably have 6 to 20 amino acids, most preferably 9 to 15 amino acids.

Preferably P + Q is less than or equal to 17, most preferably P + Q is less than or equal to 8.

Preferably X contains His.

Preferably D is Tyr.

Preferably C is an uncharged amino acid; more preferably C is Ser or Thr, most preferably C is Thr.

Preferably B is an acidic residue, more preferably B is Asp or Glu, most preferably B is Glu.

Preferably Y contains His.

Thus a preferred embodiment of the invention has the general formula

X(containing a His)-Tyr-Thr-Glu-Y(containing a His).

In a sixth aspect the invention provides antigenic peptides of 5 to 25 amino acids of general formula:

X-ABCDE-Y

wherein

A is any amino acid;

E is any amino acid;

X is any group of amino acids of length P;

Y is any group of amino acids of length Q; and

P & Q may be zero and P + Q is less than or equal to 20 and B,C,D are as defined as above.

Note that since X and Y may be absent or identical the above defined "motif" may be read in either direction in many circumstances.

Preferably the peptide is 6 to 20 amino acids, most preferably the peptide is 9 to 15 amino acids.

Preferably B is Asp or Glu, most preferably B is Asp.

Preferably A is Met.

Preferably E is His.

Preferably P + Q is less than or equal to 15, most preferably P + Q is less than or equal to 10.

Thus a preferred embodiment of the invention has the general formula X-Met-Asp-Arg-Tyr-His-Y [SEQ ID NO:33].

Compounds and peptides of the invention (either alone or as a conjugate) may advantageously be immunogenic.

For example according to the invention there is provided an immunogenic ligand which comprises a synthetic polypeptide selected from compounds of sequence SEQ ID NO:1:

TIPLWFDDEIEVMIY

and analogue and homologue derivatives thereof by virtue of one or more amino acid addition, deletion, substitution; together with terminal functional derivatives thereof; or an immunogenic ligand which comprises a synthetic polypeptide selected from compounds of sequence SEQ ID NO:2:

GDNFESYACVDTPCS

and analogue and homologue derivatives thereof by virtue of one or more amino acid addition, deletion, substitution; together with terminal functional derivatives thereof.

For example the immunogenic ligand may be the pentadecapeptide of SEQ ID NO:1

TIPLWFDDEIEVMIY

or the pentadecapeptide of SEQ ID NO: 2

GDNFESYACVDTPCS

and terminal functional derivatives thereof.

In certain embodiments of the invention the immunogenic ligand is conjugated to a carrier.

In certain embodiments of the invention the immunogenic ligand comprises a bacteriophage which encodes and is capable of expressing a synthetic polypeptide as defined above.

In a seventh aspect the invention provides an antibody produced by an immune response to an immunogenic ligand as defined above which antibody specifically recognises and binds the synthetic polypeptide of the ligand and also the group-B meningococcal Capsular Polysaccharide (CPS-B).

The invention also provides a pharmaceutical composition containing as active ingredient at least one ligand or one antibody as defined above, and optionally including an adjuvant or excipient, for use in the treatment of infection by group-B meningococcal bacteria or for use in prophylactic prevention of infection by group-B meningococcal bacteria.

This invention also provides a method of treatment or prophylaxis of infection with group-B meningococcal bacteria, which comprises administering an effective amount of a composition, antigen, ligand or antibody as defined above.

Amino acids and amino acid residues herein represent D and L amino acids, their analogues or derivatives.

The invention will now be described by way of example only with reference to the accompanying figures in which:

Figure 1 is a geometric representation of pharmacophore 1 in which position 1 indicates the ring aromatic, position 2 indicates the location of the negative charge, and position 3 indicates the location of either the hydrogen bond donor or the hydrogen bond acceptor;

Figure 2 is a geometric representation of pharmacophore 2 in which position 1 indicates the ring aromatic, position 2 indicates the location of the negative charge, and position 3 indicates the location of either the hydrogen bond donor or the hydrogen bond acceptor;

Figure 3 corresponds to the figure 1 pharmacophore wherein positions 1, 2 and 3 are represented by spheres based on the centroids of the chemical features;

Figure 4 corresponds to the figure 2 pharmacophore wherein positions 1, 2 and 3 are represented by spheres based on the centroids of the chemical features;

Figure 5 shows the pharmacophore of figure 3 superimposed with two palindromic molecular structures represented by the sequences YTE and DRY.

Figure 6 shows the pharmacophore of figure 4 superimposed with two palindromic molecular structures represented by the sequences DRY and YTE.

Figure 7 shows the pharmacophore of figure 3 superimposed with a molecular structure represented by the sequence MDRYH;

Figure 8 shows the pharmacophore of figure 4 superimposed with a molecular structure represented by the sequence MDRYH;

Figure 9 shows the pharmacophore of figure 3 superimposed with a molecular structure of the dye remazol brilliant violet;

Figure 10 shows the pharmacophore of figure 4 superimposed with a molecular structure of the dye remazol brilliant violet;

Figure 11 shows the pharmacophore of figure 3 superimposed with a molecular structure of the capsular polysaccharide of group-B meningococci;

Figure 12 shows the pharmacophore of figure 4 superimposed with a molecular structure of the capsular polysaccharide of group-B meningococci;

Figure 13 corresponds to figure 1 but shows the presence of an additional hydrogen bond donor/acceptor;

Figure 14 corresponds to figure 2 but shows the presence of an additional hydrogen bond donor/acceptor;

Figure 15 corresponds to figure 3 but shows the presence of an additional hydrogen bond donor/acceptor;

Figure 16 corresponds to figure 4 but shows the presence of an additional hydrogen bond donor/acceptor;

Figures 17 through 21 correspond to figures 5, 7, 9 and 11 but show the presence of an additional hydrogen bond donor/acceptor;

Figure 22 shows results of probing a primary synthetic peptide library with a human anti-group-B polysaccharide antibody (IgM^{Nov});

Figure 23 shows a combinational pharmacophore based on pharmacophores 1 and 2 superimposed on eachother. The resulting more detailed pharmacophore has features of both pharmacophores 1 and 2.

Figure 24 shows the pharmacophore of figure 23 and the centroids are labelled.

Figure 25 shows the absolute positions of the centroids of the pharmacophore shown in figure 23.

Figures 26 to 29 show the distance and angle constraints of the centroids shown in figures 24 and 25.

Figure 30 demonstrates that the antigenic core of the GDN... peptide (ESYACVDTPCS) maps to a combined version of the pharmacophore. Thus, critical side chains map to all of the features of this pharmacophore: E and D to the negative ionizable features; Y to the aromatic/hydrophobic feature and the hydroxyl of the Y side-chain maps (in this instance) as a hydrogen bond donor. The antigenic importance of other residues in the peptide that do not map to identified features of the pharmacophore is likely to be attributable to their effect on the conformation of

this peptide, thereby affecting the disposition of these critical side-chains.

Figure 31 depicts the mapping of the combined pharmacophore to the antigenic core region of peptide TIP..., namely DEIEVMIY. In this mapping D1 and E4 satisfy the negative ionizable features, Y satisfies the ring aromatic/hydrophobic feature, a backbone N-H providing the hydrogen-bonding element (donor in this instance).

Figure 32 depicts the mapping of methylmalonyl coenzyme-A to the combined pharmacophore. Note that all of the features are satisfied.

Figure 33 shows the less active compound, D-luciferin, of IC50 36μM fitting the combined pharmacophore. Note that in this mappinng, not all features are mapped, consistent with its lower activity.

Figure 34 depicts an alternative mapping of D-luciferin to the combined pharmacophore. As before, not all features are mapped. The ability of D-luciferin to map in two distinct modes to the pharmacophre may explain the fairly good antigenic activity of this relatively small compound.

Figure 35 shows that the polysaccharide is capable of satisfying several (4/5) structural features of the combined pharmacophore. Other mappings were found to be possible (not shown#). Surprisingly, in this particular mapping the negative ionizables fit well with alternate carboxylates of the polysaccharide (and not with adjacent carboxylates as might be anticipated) while both of the

hydrogen bonding features (having a shared point of origin) are also satisfied. Other mappings of the pharmacophore (not shown) were found to be possible, including ones in which the aromatic/hydrophobic feature was occupied by the six-membered ring of the saccharide monomer. Although this ring is not strongly hydrophobic in character, it may at least be compatible with occupying a hydrophobic site on the antibody. The existence of a plurality of mappings of the combined pharmacophore to this structure may reflect an ability of the antibody to engage multiple aspects of the helical structure of the polysaccharide, meaning that antigens identified by the pharmacophore may be particularly effective immunogens.

Figures 36 and 37 show tables illustruating the degree of reaction of antibody with peptides synthesized on membrane assessed using the method of Gao and Esnouf (1996). Scoring of reaction is as follows: '0' = no change (equivalent to signal generated with original peptide sequence); -1modest decrease; -2 strong decrease; -3 very strong decrease; # signal abolished; $ same as original sequence. Positive numbers represent an increase in signal. Deviations of 2 units or greater from the original signal are considered to be significant (i.e. 2 or -2). Blank cells indicate 'not determined'.

Figure 38 shows inhibition of binding of human anti-polysaccahride antibodies IgM Nov and 64V to solid phase poysaccharide by solution phase haptenic antigens of the diadenosine polyphosphate homologous series.

Figure 39 shows graphs of the binding of IgM Nov to Ala scanned mutants of GDN...

Figure 40 shows the list of compounds fitting the combinatorial pharmacophore and the training set for the Combinatorial pharmacophore.

(For all of Figures 3 through 12, 15 through 21 and 23 through 35 the Figures are attached in two versions: a) in black and white and b) in colour print).

To generate an appropriate antigenic peptide from a phage-peptide library an antibody is used to select phage expressing the peptide from mixtures of phage clones expressing a great variety (up to 100 million) of unique peptide sequences, essentially as described by Scott and Smith (Science, 1990). The identity of the peptide ligands is then deduced by sequencing of the recombinant DNA encoding the insert.

In the case of synthetic peptide libraries, suitable peptides can be generated most conveniently by screening of complex mixtures of peptides synthesised as pools, for example in the form of a solid phase array (Gao et al 1996 in press) as described below.

Panning of hexapeptide libraries on pIII (coat protein encoded by phage gene-III) of filamentous phage according to the method of Scott and Smith (1990) was performed using IgM^{Nov}, a well-characterised human antibody against the capsular polysaccharide of the group-B meningococci which is both bactericidal and protective in the infant

rat model. The resulting clonal population of peptide-expressing phage selected by the antibody did not however exhibit any thematic or consensus motif among the peptides sequenced (see e.g. SEQ ID NOS:12-24). Also, the selected clonal population of phage did not exhibit any evidence of specific binding to the selecting antibody on solid phase in ELISA. Further studies of the binding properties of individual phage clones demonstrated only weak specific binding of certain examples of the antibody selected clones, with signal to background ratios in the region of 2:1 or less. Such peptides, having such low avidity for the selecting antibody were not deemed suitable candidates for use in vaccine development. Moreover, the absence of thematic sequences for these peptides made it difficult to determine what physico-chemical attributes of the peptides were responsible for antibody binding, or mimicry of the polysaccharide. Moreover, the reactivity of synthetic forms of these peptide ligands in solution phase with IgM^{Nov} was weak or undetectable by competition ELISA with polysaccharide on the solid phase.

Given the failure of the phage gene-III system to generate good ligands for IgM^{Nov}, and the known requirement of anti-group-B polysaccharide antibodies to recognise a relatively large conformational feature of the polysaccharide eg. comprising several of the sialic-acid monomers (Kabat), it was deemed desirable to use larger peptides. Since the numerical equivalence of amino-acid residues with sialic acid monomers was uncertain, we chose a library of 15mers to give a margin of error. This second phage system comprised a display library system whereby

random peptides were displayed fused to the major coat protein - pVIII, derived from gene-VIII.

Selection of the hybrid phage random library of 15mers with IgM^{Nov} gave rise to a polyclonal population of selected phage which did exhibit specific binding to the selecting antibody on ELISA (in contrast to experience with the 6mer library in gene-III).

Binding studies on the isolated phage clones revealed two which gave strong signal to background ratios, of the order of 10:1 on ELISA. These phage clones were quite different in primary sequence, one containing a putative disulphide bond (GDN...:SEQ ID NO:2), the other (TIP...:SEQ ID NO:1) exhibiting a strong helical propensity according to secondary structure predictions. Despite the superior binding characteristics of these phage clones isolated from the 15mer libraries in gene-VIII, the dearth of sequences isolated in these experiments and their lack of a common motif still failed to identify what physicochemical attributes (e.g. what residues, sequences etc.) were responsible for peptide binding to the antibody, or for antigenic mimicry of the polysaccharide. Moreover, modelling of the peptides using energy minimisation techniques also failed to identify candidate structural features which might explain their binding to an anti-polysaccharide antibody.

Given the strong specific binding of the selected phage clones expressing 15mer ligands for IgM^{Nov}, even under conditions of monovalent display, it was surprising to find that the synthetic versions of these peptide

sequences exhibited only weak activity in competitive ELISA where the solution phase peptides were tested for their ability to inhibit antibody-binding to solid phase polysaccharide. Thus, although the phage-displayed 15mers were good ligands, their antibody binding was dramatically weakened once dissociated from the context in which they were selected (i.e. that of the phage coat). These findings of the context-sensitive antigenicity of phage gene-VIII displayed polysaccharide-mimetic peptides suggest the imposition of significant structural constraint by the phage gene-VIII coat environment, and concur with much of our experience with antibodies against proteinaceous antigens of infectious agents. These findings further suggest that that the structure of peptides mimicking the group-B polysaccharide, rather than merely the residues they contain or the sequence in which these residues occur, is fundamentally important to their polysaccharide mimicry. However, these findings give little clue as to what that structure is. Studies were therefore initiated with synthetic peptide libraries in order to gain insight into the amino-acid residues, sequences and peptide structures that determine peptidic mimicry of the group-B polysaccharide.

Array libraries of synthetic peptides were constructed. Such libraries are constructed as an array of spots on a membrane - each spot conforming to a prescribed generic formula, e.g. XOXOXXXX [SEQ ID NO:3] - where 'X' represents a mixture of all 19 proteinogenic amino acid residues (excluding cysteine) and where 'O' represents a fixed position in the sequence wherein the identity of the amino acid residue at that position is known. By arranging

the spots in a 19 x 19 grid it is possible to explore all possible permutations (361) of the fixed residues, i.e. XAXAXXXX [SEQ ID NO:4], XAXDXXXX [SEQ ID NO:5] etc., where each spot contains over 1 million unique explicit peptides and the library contains about 2 x 10^{13} sequences in all.

The probing of such an array library with Nov serum (containing IgM^{Nov}) followed by an anti-mu-chain specific peroxidase conjugate revealed selective staining of certain spots upon exposure of the blot to X-ray film in the presence of a luminescent peroxidase substrate. The strongest of these signals represented the intersections of column Y with row E (representing XYXEXXXX [SEQ ID NO:6]) and of column D with row Y (representing XDXYXXXX [SEQ ID NO:7]). Interestingly, these signals provide a degree of mutual confirmation since they exhibit an imperfect form of palindromy -YXE- being analogous to -DXY- (D and E each have negatively charged side chains). Moreover, the truly palindromic representations of the strongly recognised sequences -YXE- and -DXY- (i.e. spots containing -EXY- and -YXD-) also gave rise to positive signals, albeit weaker ones. Refer to figure 24. These findings clearly implicate the residues Y and D/E as critical for the binding the anti-group-B polysaccharide antibody IgM^{Nov} to peptides. Further studies with secondary synthetic libraries (see below) also demonstrate that these signals are indeed attributable to IgM^{Nov}, since they are abolished by preabsorption of the serum with Colominic acid (i.e. α-(2,8)-linked polysialic acid, the common capsule material of *E.coli* K1 and the group-B meningococci). Moreover, these findings also demonstrate stereospecificity of binding, since YXE is not

stereochemically equivalent to EXY and DXY is not stereochemically equivalent to YXD.

In the above studies with a primary synthetic peptide library we chose arbitrarily to fix the spacing and distribution of fixed residues in the library template in the format XOXOXXXX [SEQ ID NO:3], since our previous studies with anti-protein antibodies (BG) had proven this spacing to be suitable. Since there was no priori reason to suppose that such a format would be optimal for the anti-polysaccharide antibody, we also chose to explore alternative spacing and positioning of the fixed residues in the generic template to determine the optimal spacing and positioning for each permutation of fixed residues.

Further libraries were therefore constructed to explore alternative spacing and positioning for all 361 permutations of fixed residues. Templates of the following generic formulae, OOXXXXXX [SEQ ID NO:8], OXOXXXX [SEQ ID NO:9], XOXXOXXX [SEQ ID NO:10] and XXXOXXOX [SEQ ID NO:11], were constructed exploring spacing of 0, 1 and 2 with various positioning, in a search for further binding motifs. This revealed the following additional binding motifs for IgM^{Nov}:

1. REXXXXXX [SEQ ID NO:12]
2. TDXXXXXX [SEQ ID NO:13]
3. TEXXXXXX [SEQ ID NO:14]
4. HXDXXXXX [SEQ ID NO:15]
5. DXHXXXXX [SEQ ID NO:16]
6. LXHXXXXX [SEQ ID NO:17]
7. XDXHXXXX [SEQ ID NO:18]

8. XXDHXXXX [SEQ ID NO:19]
9. HXXEXXXX [SEQ ID NO:20]
10. XHXXEXXX [SEQ ID NO:21]
11. XDXXHXXX [SEQ ID NO:22]
12. XXHXXEXX [SEQ ID NO:23]
13. XXXHXXEX [SEQ ID NO:24]

This study confirmed observations made earlier in this document that histidine (H) was strongly implicated as a determinant of binding, with certain strict provisos. Also, the simultaneous presence of an negative (e.g. acidic) residue is mandatory (D or E), with a preferred spacing of two residues between the negative residue and H, although a spacing of one residue is tolerated. Whilst tyrosine (Y) plus a negative (e.g. acidic) residue were found to be important, palindromy was either not tolerated, or was tolerated only poorly. Thus, whereas HXXE [SEQ ID NO:25] was positive, EXXH [SEQ ID NO:26] was not. Likewise although DXXH [SEQ ID NO:27] was positive, HXXD [SEQ ID NO:28] was not. Furthermore, when separated by a single residue, this study reveals poorly tolerated palindromy of HXD which is preferred over DXH. Interestingly however, neither HXE nor EXH was tolerated.

These findings demonstrate stringent requirements for the spacing of H with a negative (e.g. an acidic) residue as determinants of binding. They also further underscore the stereochemical specificity of the interaction of the anti-polysaccharide antibody with peptide antigens. Further studies described below using secondary synthetic libraries, confirm and extend the findings of the primary and secondary library studies in implicating histidine and

tyrosine as determinants of binding in combination with acidic and other amino acids at specified positions. These latter studies further demonstrate that the stringency of spacing of histidine and the acidic side chain of glutamic or aspartic acid becomes less important when other residues are allowed to make a major contribution to binding.

Secondary synthetic libraries were constructed according to the spot-method (Gao et al in press) based upon the major leads found in the primary library studies, to give templates of the following generic formulae: XYXEXXXX [SEQ ID NO:29] and XDXYXXXX [SEQ ID NO:30]. A marked reactivity of IgM^{Nov} was observed with histidine-containing peptides in each of these libraries. Comparison of these results with those of the primary library studies (above) demonstrate that this histidine-dependent reactivity is contingent upon the presence and appropriate distribution of both tyrosine and an acidic residue, since most of the 37 histidine-containing pools represented by spots in each of the five primary libraries (which explored spacing etc: see above) were unreactive with the antibody. With this proviso, in general terms, the requirement for histidine was largely position-independent in these secondary 8-mer libraries. The single exception to this rule was a preference for histidine at position 4 in the secondary library of template XDXYXXXX [SEQ ID NO:30](i.e. XDXYHXXX [SEQ ID NO:31] (which contains a motif -DXYH- SEQ ID NO: 32). Figure 25 shows the results of probing two secondary synthetic peptide libraries of generic formulae XYXEXXXX (upper panel) and XDXYXXXX (lower panel) with a human anti-group-B polysaccharide antibody. Not all signals

were inhibited by preabsorption of the antibody with polysaccharide (Colominic acid), the ones which were strongly inhibited are indicated. The preferred linear peptides can be inferred from this data as -YT/sE-[H] (upper) where T/s represents a preference for threonine and a tolerance of serine. The brackets around "H" represent unspecified position in the sequence. The dashes represent occurrence internally in the sequence. The other preferred peptide was MDXYH, where the presence of histidine at position 5 was preferred. This finding confirms the observations made with the primary libraries which explored positioning and spacing of the fixed 'O' residues. These studies implicated -DXXH- [SEQ ID NO:27] as a positive motif, consistent with the XDXYHXXX [SEQ ID NO:31] peptide found in the secondary library study.

The findings of the secondary synthetic library studies which used linear peptides can be summarised as two motifs, namely: -YT/sE-[H] and MDXYH, where dashes indicate but do not exclude occurrence non-terminally in a peptide sequence, and where parenthesis indicates non-specified position in the sequence.

The identified motifs are unexpectedly short - given the requirement of IgM^{Nov} to recognise higher oligomers of sialic acid (9-unit oligomers being optimal). This may be because these peptides bind in an extended conformation, whereas the polysialic acid binds in a helical conformation (Brisson et al). Alternatively, they may reflect the binding of the peptides to part only (i.e. a subsite) of the antigen-combining surface of the antibody.

Since the studies with synthetic peptide libraries used IgM^{Nov} serum rather than purified antibody, signals emanating from other serum IgM antibodies may have been attributed erroneously to IgM^{Nov}, or perhaps to specific binding of the anti-mu-chain conjugate to peptide motifs in the libraries. In order to demonstrate the specificity of signals obtained from the synthetic libraries, two types of control experiment were performed. First, a reagent blank was used, omitting the Nov serum. Under these conditions, no signal was generated. Secondly, serum was preabsorbed with Colominic acid in solution phase and the serum used to probe libraries. Preabsorption with Colominic acid resulted in the abolition or near-abolition of strong signals, leaving numerous weak signals uninhibited. In this way it was possible to discern which signals were genuinely attributable to the anti-group-B polysaccharide antibody (IgM^{Nov}).

We have demonstrated that the preferred polysaccharide mimetic peptide motifs of IgM^{Nov}, with respect to linear peptide sequences, are MDRYH [SEQ ID NO:33] and MDHYH [SEQ ID NO:34]. However, these data give no indication whether the motif or related motifs are peculiar to IgM^{Nov} or whether they represent a more-general characteristic of anti-group-B polysaccharide antibodies in general. We, therefore, tested other paraproteins which had been identified by us previously by random screening of paraprotein containing sera as reactive with the group-B polysaccharide. Three such sera were tested against the 19-member DXYH [SEQ ID NO:32] set of motifs recognised by IgM^{Nov} compared to two control sera that contained paraproteins which were not reactive with Colominic acid.

Each of the three Colominic acid reactive paraproteins were found to react with several members of the 19 member DXYH [SEQ ID NO:32] family of peptides, whereas the control paraproteins did not react with any of the 19 peptides. These data demonstrate that the DXYH [SEQ ID NO:32] motif is not unique to IgM^{Nov} but represents a more-general property of human anti-group-B polysaccharide antibodies.

These relatively small motifs identified from the linear peptide libraries may indicate that small linear peptides of the specified sequences are adequate to occupy the antigen-combing site of anti-group-B polysaccharide antibodies fully. Alternatively, they may indicate that the flexibility of linear peptides does not allow the techniques used to specify structural attributes of a larger antigenic structure, because appropriate conformers may be too low in abundance to allow this.

We sought means to extract further structural information from the data generated from the synthetic peptide libraries. Moreover, our hypothesis and the demonstration that IgM^{Nov} can react with such materially diverse antigens as polysaccharide and peptides, led us to consider whether structural inferences made from our data could lead to the identification of further chemical species (i.e. non-peptide, non-saccharide) which would comprise potential vaccine components or further define the stereochemical attributes of a suitable antigen. A conformational model was therefore built using computational methods based on structure activity data for

a series of linear peptides reactive with IgM^{Nov}, as follows.

Linear peptides convey little structural information. However, we postulated that by identifying consensus structural features of families of peptide ligands that bind to IgM^{Nov}, taking into account their relative binding activity, it should be possible to identify the epitomising structural features of the antigenic structure most preferred by the antibody. Such procedures have been used before to identify 'pharmacophores' that epitomise the ideal structural features of drugs, and which can be used as structural templates to search databases of molecular structures for novel drug leads. However, such techniques have not previously been applied to antigens or antibodies, nor (to our knowledge) have peptide libraries been used to generate pharmacophores.

A pharmacophore is a constellation of physicochemical features (charge, hydrophobicity, etc.) defined by a map of their relative positions and absolute distances in three-dimensional space, which epitomises the structural attributes required for a compound to have a particular pharmacological activity. The validity of a pharmacophoric hypothesis can be tested experimentally by demonstrating that exemplars of that hypothesis have the predicted attributes in assays of binding or biological activity. We now describe the application of this concept to antibody-antigen interaction. In the case of antibodies, there is no "activity" in a strict pharmacological sense, although the antigenic equivalent of a pharmacophore would be predicted to have immunogenic

activity *in vivo*. With this proviso, the pharmacophoric description of the group-B polysaccharide which follows relates to antibody-antigen binding without implied pharmacological activity.

Of all possible pharmacophores that can occupy three-dimensional space, there are two related pharmacophore classes that are both statistically significant (in the sense that they describe the structure-activity relationship of the training set of peptide ligands for IgM Nov with a correlation that is statistically significant) and also predictive of antigenic (and potentially immunogenic) ligands, of IgM^{Nov}. These pharmacophores were generated in the following way.

Data for pharmacophore generation was generated from the primary and secondary linear octameric peptide libraries (of general formula XOXOXXXX) referred to earlier in this document. A training set of 19 'virtual' tripeptides was constructed from the 'OXO' region of the octameric peptides. The peptides comprising the training set are described in the table of figure 40. Conformational models that emphasized coverage over a 20 kCal range above the estimated global minimum were generated in Catalyst for each tripeptide. [Smellie, A, Teig, S.L. & Tobin,P. J.Comp.Chem. 1995 16: 171-187; Smellie, A. Kahn, S.D., Teig S.L. J. Chem. Inf. Comp. Sci 1995 35:285]. In lieu of dissociation constant values for the tripeptide ligands, 'relative affinity' scores were generated subjectively - based on the time and intensity of exposure of X-ray films exposed to the luminescent membrane-bound samples.

In order to maximise the effectiveness of pharmacophoric searching of this dataset, the feature constraints in the table below were applied.

Feature Name	Ring Aromatic	H-bond Donor	H-bond Acceptor	Negative Ionizable	Total
minimum	0	0	0	0	1
maximum	5	5	5	5	5

Inter-feature spacing was set to 1.5 Angstroms. The mapping coefficient was set to 1, in order to force topologically similar molecules to map the generated hypothesis in similar ways. All other parameters were set to the manufacturer's default values for revision 3.0 of of the software (i.e. Catalyst).

Each of these models consisted of at least three common chemical features: a Ring Aromatic, a Negative Charge and a Hydrogen Bond Acceptor or Donor feature. (The 'common' hydrogen bonding features of the two hypotheses have shared points of origin within the tolerances of these features, although their projected points differ). The features of the pharmacophores are further defined below:

A hydrogen bond acceptor feature matches the following atom types or groups of atoms which are surface accessible;

- sp or sp^2 nitrogens that have a lone pair and a charge less than or equal to zero
- sp^3 oxygens or sulphurs that have a lone pair and charge less than or equal to zero
- non-basic amines that have a lone pair

A hydrogen bond donor feature has the same chemical characteristics as the hydrogen bond acceptor except that it also includes basic nitrogen. There is no exclusion of electron-deficient pyridines and imidazoles. This feature matches the following atom types or groups of atoms;

- non-acidic hydroxyls
- thiols
- acetylenic hydrogens
- NH moieties (except tetrazoles and trifluoromethyl sulfonamide hydrogens)

A negative charge feature is defined as a negative charge not adjacent to a positive charge.

A ring aromatic feature is defined as an aromatic moiety which may be replaced by amino acid residues having hydrophobic character. Tyrosine may be categorised as "uncharged polar", but it is also somewhat hydrophobic. Thus, according to the present invention this feature matches the following group: methionine, alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and tyrosine.

The hydrogen bond acceptor is represented by a vector function consisting of two spheres. The smaller sphere (at least 1.6Angstroms radius up to 2.6 Angstroms) defines the centroid of the hydrogen bond acceptor on the ligand while the large sphere (at least 2.2 Angstroms radius up to 2.6 Angstroms) defines the projected point of the hydrogen bond acceptor from the receptor. These two spheres are at least 3.0 Angstroms apart. Similarly, the hydrogen bond

donor is represented by a two sphere vector function as above.

The ring aromatic is represented as two equal size spheres (at least 1.6 Angstroms radius up to 2.0 Angstoms) whose centroids are 3.1 Angstroms apart. One sphere corresponds to the position of an aromatic ring moiety and the other to the projected point of the electron pi stacking of the aromatic ring system.

The negative charge is represented by a sphere at least 1.6Angstroms radius (up to 2.6 Angstoms). The tolerances on all distances between these features is +/- 0.5 Angstroms and the geometric angles +/- 20 Degrees.

The distances and angles are shown in figures 1 and 2.

Position 1 indicates the ring aromatic, position 2 indicates the first and second possible locations of the negative charge. Position 3 indicates the location of either the hydrogen bond donor or hydrogen bond acceptor. In addition to these 3 key chemical features there is a further optional feature that may enhance selectivity in screening for polysaccharide mimetic ligands for IgM^{Nov}. Position 4 indicates the position of a possible additional hydrogen bond donor/acceptor (i.e. an optional extra hydrogen bond donor/acceptor feature).

To better represent these pharmacophores, reference should be made to figures 3 and 4.

Once a pharmacophoric model has been developed it is possible to evaluate the correlation between the pharmacophore and any selected molecular sequence/structure.

For pharmacophore number 1 containing three key chemical features figure 5 demonstrates the fit of the two palindromic molecules of sequence YTE and DRY; figure 7 demonstrates the fit of the 5mer peptide of sequence MDRYH. For pharmacophore 2 the fit of the same molecules in the alternative pharmacophoric model is shown in figures 6, and 8. The figures 19 and 20 show the same two molecules fitted to pharmacophore 1 which includes the optional additional fourth chemical feature of another hydrogen bond donor/acceptor.

The predictive accuracy of the pharmacophoric model of the antigen was evaluated by comparing the observed activity of peptide ligands versus that predicted by the model. The resulting correlation (r=0.95) demonstrated that the data are highly consistent with the pharmacophoric model.

In order to validate this hypothetical pharmacophoric description of antigenic ligands for IgM^{Nov}, the model was used to search structural databases of available chemicals (i.e. the Available Chemicals Directory). Several of the top ten hits were then screened by competition ELISA using IgM^{Nov}. Two of these compounds, Direct Red 80 and remazol brilliant violet were found to be antigenically active, causing complete inhibition of antigen binding by IgM^{Nov}, thereby validating the model. It follows that compounds such as remazol brilliant violet and Direct Red 80 which

conform to this structural model are potential candidates for use in vaccine development. Reference should now be had to figures 11, 12 and 22 which respectively show remazol brilliant violet dye superimposed on pharmacophore 1, pharmacophore 2, and pharmacophore 1 with the additional fourth chemical feature.

As a further confirmation of the validity of the pharmacophoric models represented by the patterns of pharmacophore 1 and pharmacophore 2, a structural representation of the CPS-B molecule was superimposed respectively on pharmacophore 1, pharmacophore 2 and pharmacophore 1 including the fourth chemical feature. The results are shown respectively in figures 13, 14 and 23. It is notable that the fit is good in each case.

The synthetic peptides can be expressed on the surface of filamentous bacteriophage using standard techniques for phage display such as those described by Perham et al (WO92/07077) and Greenwood et al (Greenwood J., Willis A. E. and Perham, R. N. 1991. J. Mol Biol. 220. 821-827) to produce peptide ligands in the correct structural orientation. The constructs can then be used to evaluate the potential of the peptide ligands to mimic the capsular polysaccharide.

The above studies on phage peptide ligands and synthetic peptide ligands for IgM^{Nov} and other human anti-group-B polysaccharide antibodies have identified peptide antigenic mimics of the capsular polysaccharide, and have revealed in some detail the amino-acid residues, sequences and structures of these peptides, and the essential

features of non-peptide non-saccharide chemical entities, which are necessary for mimicry of the group-B meningococcal polysaccharide. The data described so far infer, but do not prove, that peptides (and perhaps other novel chemical entities) with these properties will be capable of eliciting anti-polysaccharide antibodies when formulated appropriately as phage-peptide or peptide-protein conjugates, or with some other form of immunogenic carrier to provide T-cell epitopes.

To test whether the peptides identified are immunogenic as well as antigenic for the group-B polysaccharide, phage clones expressing the sequences TIPLWFDDEIEVMIY [SEQ ID NO:1] (referred to as "TIP...") and GDNFESYACVDTPCS [SEQ ID NO:2] (referred to as "GDN...") were used to immunise rats, and the resulting sera were tested for anti-polysaccharide antibodies. Groups of six rats were immunised subcutaneously with 12.5 microgram doses of the phage clones, at weeks zero, 2, 4, 6 and bled at intervals. No adjuvant was used. The results of testing the pre-immune and week 8 sera show that strong responses to the polysaccharide were observed in each of the immunised groups, particularly that group immunised with the GDN... phage which was the better antigen when tested for antigenicity.

These findings demonstrate that the peptide ligands we have identified are immunogenic for the polysaccharide as well as being antigenic mimics of the polysaccharide. We have further demonstrated that antibodies in the rat sera were specific for the polysaccharide, since preabsorption

of the serum with solution phase polysaccharide diminished the signal in every case.

Further Characterisation of Phage Library Derived Antigenic Ligands of IgM Nov

i) Analysis of peptide GDN... by Site-Directed Mutagenesis on Phage and by Residue-Substitution of Synthetic Peptides.

The phage clone expressing peptide GDN... was subjected to Ala-scan mutagenesis according to standard methods wherein the DNA encoding particular amino-acids in the peptide was substituted for a codon representing alanine. Phage were propagated in E.coli and purified by three rounds of precipitation with polyethylene glycol. The binding of unaltered and mutated phage clones was assesed in a solid phase electrochemiluminescent (ECL) assay using an Origen analyser (Igen). Magnetic beads were coated with a polyclonal anti-phage (anti-M13) antibody and used to capture phage from solution. The phage-coated beads were then exposed successively to IgM Nov, and to an anti-mu chain antibody labelled with an electrochemiluminescent tag according to manufacturers instructions (Igen). Figure 39 shows that the GDN... expressing phage clone ('GDN') gave a concentration-dependent signal in this assay. In these experiments all resides of the peptides, except proline (at position 10) were systematically replaced by alanine.

It is evident from these studies that there is a clear distinction between residues which tolerate and those

which do not tolerate substitution by alanine. Thus, the first four residues of this peptide (GDNF) are seen to be unimportant to antigenicity. However, each of the cysteine residues at positions 9 and 14 (C9, C14) were found to be essential to binding, demonstrating the importance of the short disulphide loop (CVDTPC) to antigenicity. Consistent with these observations, residues within this loop, i.e. V10 and D11 were not tolerant to alanine substitution, although T12 could be replaced by alanine (relatively conservative substitution). Also, E5 and Y7 were found to be essential. It is noteworthy that the acidic residues D,E and tyrosine (Y) were found to be essential to the binding of IgM Nov this peptide on phage, just as was the case with the synthetic peptide libraries, providing mutual confirmation of the results of these technically diverse approaches. In these studies however, it was found not to be possible to substitute proline for alanine.

Next, a synthetic form of the peptide GDN... was subjected to a 'substitution net analysis' wherein all of the proteinogenic amino-acids were systematically replaced at certain positions of the peptide. The results are presented in the table of figure36 and refer to the region of the GDN... peptide beginning at E5 such that the peptide now reads ESYACVDTPCS, with E now referred to as E1. The results of this study are in broad agreement with the phage mutagenesis studies described above. Precise agreement between these two diverse techniques (phage disply versus synthetic peptides on membranes) would not be expected, given the differing contexts of presentation of the peptide in these two systems, and the likely influence of context upon conformation of the peptide.

Using the synthetic peptide approach, substitution of the single proline residue of this peptide was accomplished without difficulty. With the exception of serine, no other residue was well tolerated in place of proline, demonstrating the importance of this residue to the antigenic structure of the peptide. These data also demonstrate strikingly that D11 is important for binding, all substitutions except alanine not being tolerated. V10 was intolerant to alanine substitution by this method, although numerous other residues could be tolerated at this position. Although Y7 was found to be tolerant (by this method) to alanine substitution, its importance for antigenicity was confirmed by the finding that virtually all other substitutions attenuated or abolished binding.

According to this latter analysis, using peptides synthesized on membranes, certain residues were suggested to be preferred over the original sequence. These were D in place of E at position 1 of this 12-mer, giving DSYACVDTPCS. Also, at position 2, A was preferred over the original S giving EAYACVDTPCS.

ii) Analysis of peptide TIP... by Residue-Substitution of Synthetic Peptides.

Peptide TIP... was subjected first to a 'window-scan' analysis whereby peptides were made on membrane (as above) representing subsequences comprising residues 1-8, 2-9, 3-10 etc.of this 15mer peptide. This analysis (not shown) identified two overlapping peptides of sequence DDEIEVMI and DEIEVMIY respectively to be antigenically active, localising the antigenicity of the 15mer peptide TIP... to

a smaller 9-mer sequence (i.e. DDEIEVMIY). To ascertain precisely which residues of this peptide were most important for antigenic mimicry of the polysaccharide, a replacement net was constructed wherein each of the 9 residues of the sequence DDEIEVMIY were replaced by 9 of the proteinogenic amino acids (namely A,D,E,H,K,N,P,S,V,W,Y). The results are summarised in the table of figure 37.

It was found that most of these residues (i.e. all except D1) were highly intolerant to substitution, whereas the first residue (D1) could be replaced by any of several amino acids of diverse character, Thus, although the acidic and hydrophillic residue 'E' (similar to the original D) was preferred over D at the D1 position, so too was valine (a hydrophobic branched chain amino acid). Even tryptophan (a large aromatic hydrophobe, very different in character to the original D) was well tolerated at this position. The most antigenic part of peptide TIP can be seen from this analysis to be 'DEIEVMIY'. It is notable that this sequence contains both tyrosine and the acidic residues D and E as important features, as for the peptides identified by the synthetic library studies and as in the case of the GDN... peptide (above). Thus, the substitution net of the 15mer phage-derived peptide TIP... demonstrates the existence of a core region which is highly optimised for antibody binding, namely DEIEVMIY, being surprisingly intolerant of substitution. Most notably this peptide is richly provided with residues capable of fulfilling the requirements of the 'combined pharmacophore' description of the epitope (i.e. hydrophobic/aromatic and acidic residues). It is

likely therefore that various conformations or aspects of this peptide can mimic the polysaccharide epitope. This may explain why this peptide was selected during phage display as a favoured antigenic sequence by the antibody.

As was the case with the GDN... peptide above, it was found in this analysis of the TIP... peptide to be possible to enhance the antigenicity of the original peptide. Thus, the non-conservative substitution of E3 with proline (P an uncharged cyclic *imino* acid) to give the sequence DDPIEVMIY gave rise to an increase in reactivity with the anti-polysaccharide antibody. Also, replacement of the penultimate I (I8) with Y was found to increase antigenicity modestly. Although such a modest increase may not have been significant, this finding demonstrates at least that tyrosine is tolerated at this position.

Generation of a 'Combined Pharmacophore'

The two alternative pharmcophores were found to be identical except for the positioning of a negative ionizable feature. According to their descriptions, the negative ionizable feature could be in one of two alternative positions. However, another interpretation of these findings is that the antigen-binding requirements of the antibody could be satisfied better by a molecule which would provide the common features of these two hypotheses, but in addition, simultaneously satisfying *both* of the negative ionizable features suggested originally to be alternatives.

In order to avoid generating a combined hypothesis which was too complex to search or too computationally laborious to generate, the optional hydrogen bond donor feature (common to both hypotheses) was discarded. The two constituent hypotheses were superposed using the root-mean square fitting algorithm of Catalyst to generate the combined pharmacophore. Thus, the common ring aromatic feature, the two negative ionizables (originating separately from the different constituent pharmacophores), and the H-bond donor and acceptor features (having differing vectors - yet a common centroid point of origin) were combined to generate a new 5-feature hypothesis. The combined hypothesis was used to search the Available Chemicals Directory, resulting in only 2 hits, one of which transpired to be the most active compound yet identified (i.e. methylmalonyl coenzyme-A at an IC50 of 7μM).

In order to increase the catchment of the combined pharmacophore to allow the identification of further active compounds, the tolerance on each of the two negative ionizable features of the 5-feature combined pharmacophore was increased by 1 Angstrom. This resulted in recovery of 11 hits from the ACD, illustrated in the table of figure 40. The results of searching the ACD with the various hypotheses is summarized below.

From a database search of the ACD, 26 and 171 compounds were recovered as 'hits' (i.e. mapping all features of the pharmacophoric hypothesis) for the two alternative constituent pharmacohphores, respectively, when searched independently. These compounds were subject to a further

screen to avoid duplication and to eliminate potentially reactive species, a representative sample set of these compounds was tested by immunoassay. With hypothesis 1, of 9 compounds procured and tested, 6 were antigenically active (67%). Similarly, with hypothesis 2, all 12 compounds procured were found to be antigenically active. (i.e. IC50's less than 1mM).

We tested several of the compounds retrieved by the combined pharmacophore (with expanded negative ionizable features - see above), and found a surprisingly high fraction of them to be antigenically active. Thus, of 11 compounds identified, 8 were procured and 4 of these were strongly antigenically active (IC50 < 200μM), the remaining ones weakly so (>410 μM). The strongly active compounds were: nicotinamide 1, N6 ethanoadenine dinucleotide phosphate, P1,P5-di (adenosine-5') pentaphosphate, nicotinamide hypoxanthine dinucleotide phopshate and methylmalonyl coenzyme A (IC50 7μM). This is a remarkably high hit rate (50%) compared to the rates expected for random screening of compounds (<1%) and therefore represents strong evidence for the validity of the more-detailed 'combined' form of the pharmacophoric hypothesis. Notably, the non-phosphorylated form of N6 ethenoadenine dinucleotide phosphate was markedly less active (IC50 of 150μM) than its phosphorylaed counterpart (IC50, 11μM) emphasizing the importance of having two negative charges.

Relative Importance of Negative Ionizables Versus Other Features of the 'Combined Pharmacophore'

In order to assess whether the posession of a pair of appropriately spaced negative ionizables was pre-eminently important to antigenicity, or whether other aspects of the pharmacophoric description comprising the 'combined pharmacophore' were comparbly important, an investigation was carried out using the di-adenosine polyphosphate series of compounds. A search with the combined pharmacophore and immunosassay had identified P1', P5' diadenosine polyphosphate as an antigenically active compound. Advantage was taken of the existence of an homologous series of these compounds ranging from the triphosphate to the hexaphosphate. Figure 38 illustrates a comparison of the antigenic potency of this family of compounds in a hapten inhibition assay (the assay used to determine IC50 values for haptenic antigens). It is evident from this figure that although all of the compounds (i.e. tri- up to hexa-phosphate) were fully antigenically active, there was a clear gradation of potency - increasing up to the pentaphosphate and being increased only marginally on moving up to the hexaphosphate homologue.

These observations with the diadenosine polyphosphates emphasize that although the two negative ionizable features of the hypothesis are important, yet other features of the hypothesis are also important. Thus, in ascending this homologous series, the pentaphosphate is the first compound capable of spanning the two negative ionizable features. Nevertheless, the tri and tetra phosphates were also active, although less so, despite being unable to satisfy simultaneously both negative ionizable features. The fact that these latter compounds

were active demonstrates that the satisfaction of one only of the two negative ionizable features is sufficient for antigenicity, provided that the other features of the hypothesis are satisfied. (Incidentally, it appears that the ring aromatic and hydrogen bonding features of the pharmacophore are satisfied in this family of compounds by the adenine moiety, and not as intuition might suggest - by the ribose emulating the saccharide moieties of the polysaccharide).

It is also evident from the figure depicting the comparative potency of the diadenosine polyphosphate series (Figure 38) that these compounds are highly specific for the antibody IgM_{Nov}. In contrast, the polysaccharide binding of antibody 64V (an isotype matched human paraprotein antibody also recognizing the group-B meningococcal polysaccharide) was not inhibited at all by these compounds in the range tested.

A striking feature of the small molecules identified by the combined pharmacophore was that their 'affinities' (i.e. IC50's) approximate that of the polysaccharide itself (i.e. IC50 for colominic acid was found to be 1.8μM). This is remarkable given the fact that the sialic acid monomer, of which this poymer is comprised, was found to be completely inactive in these assays (i.e. IC50 >> 1.0 mM). It is also remarkable given the valency advantage of colominic acid as an inhibitor, relative to the monovalent haptenic ligands identified from pharmacophore searching. Thus, with a degree of polymerisation of n=30 the polymer has an avidity advantage, such that it can

simultaneously express the conformational epitope several times over.

EXAMPLE 1

A recombinant filamentous bacteriophage (fd) clone expressing the sequence GDNFESYACVDTPCS [SEQ ID NO:2] (GDN...) fused to the N-terminal of the gene-VIII protein was purified by caesium chloride gradient sedimentation and injected subcutaneously in 12.5 microgram doses into six Wistar rats. This procedure was repeated for each of the six rats a further two times at 2-week intervals, and serum samples were taken at intervals. In order to test preimmune and week 8 post-immunisation sera for reactivity with colominic acid (alpha 2-8 linked polysialic acid, the capsular polysaccharide of *E. coli* K1 and the group-B meningococci), polystyrene ELISA plates coated with colominic acid were incubated first with serial dilutions of the sera and secondly with a species-specific anti-IgG alkaline-phosphatase conjugate, and finally developed with a chromogenic substrate for alkaline phosphatase. There was a highly significant increase in the assay signal (absorbance) in the post-immunization bleed, demonstrating the elicitation of IgG class antibodies against the polysaccharide. The IgG class of the antibodies is particularly significant, since it demonstrates 'class-switching' of the immune response, a hallmark of immunological memory. It is also significant that this response was obtained in the absence of adjuvant, since it demonstrates that the phage construct is particularly immunogenic for this notoriously weak antigen (i.e. the group-B polysaccharide).

Spurious signals can be generated in ELISA tests via the non-specific binding of antibodies to solid phase

components other than the antigen (e.g. the polystyrene itself or the blocking agent used to minimise non-specific binding to the polystyrene). These signals may be distinguished from specific signals, because only the latter are inhibited by preabsorption with colominic acid in solution phase. We have demonstrated that the binding of phage-peptide elicited antibodies by clone GDN... can be inhibited substantially by solution phase colominic acid, verifying the specificity of these phage-peptide elicited antibodies, and confirming their immunogenicity for the polysaccharide.

EXAMPLE 2

A second phage clone TIPLWFDDEIEVMIY [SEQ ID NO:1] (TIP...) was also used to immunise rats, precisely as described above (EXAMPLE 1). We have demonstrated that this phage clone also elicited anti-polysaccharide antibodies which were inhibited by solution phase polysaccharide.

EXAMPLE 3

A further peptide ligand according to the inventurn is GECPTGSLLLDGLYC [SEQ ID NO: 38]. This peptide ligand has tyrosine (Y) and glutamic acid (E) residues brought into proximity by means of a disulphide bond.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

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(B) STREET: 321 CAMBRIDGE SCIENCE PARK

(C) CITY: CAMBRIDGE

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(G) TELEPHONE: 01223 423333

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(ii) TITLE OF INVENTION: Meningococcal Vaccine

(iii) NUMBER OF SEQUENCES: 38

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Thr Ile Pro Leu Trp Phe Asp Asp Glu Ile Glu Val Met Ile Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Gly Asp Asn Phe Glu Ser Tyr Ala Cys Val Asp Thr Pro Cys Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION:2

(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION:4

(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Xaa Ala Xaa Ala Xaa Xaa Xaa Xaa

1 5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Xaa Ala Xaa Asp Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Xaa Tyr Xaa Glu Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Xaa Asp Xaa Tyr Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:1

(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION:2

(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

1 5

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION:1

(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION:3

(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:2
(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:5
(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:4
(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:7
(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Arg Glu Xaa Xaa Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Thr Asp Xaa Xaa Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Thr Glu Xaa Xaa Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

His Xaa Asp Xaa Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Asp Xaa His Xaa Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Leu Xaa His Xaa Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Xaa Asp Xaa His Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Xaa Xaa Asp His Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

His Xaa Xaa Glu Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Xaa His Xaa Xaa Glu Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Xaa Asp Xaa Xaa His Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Xaa Xaa His Xaa Xaa Glu Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Xaa Xaa Xaa His Xaa Xaa Glu Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

His Xaa Xaa Glu
1

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Glu Xaa Xaa His
1

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Asp Xaa Xaa His
1

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

His Xaa Xaa Asp
1

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Xaa Tyr Xaa Glu Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Xaa Asp Xaa Tyr Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Xaa Asp Xaa Tyr His Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Asp Xaa Tyr His
1

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Met Asp Arg Tyr His
1 5

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Met Asp His Tyr His
1 5

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION:4

(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION:6

(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys
1 5

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Xaa Xaa Tyr Xaa Asp Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

Arg Glu Tyr Glu Asp Met Met
1 5

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Gly Glu Cys Pro Thr Gly Ser Leu Leu Leu Asp Gly Leu Tyr Cys
1 5 10 15

Claims

1. A pharmaceutical compound which includes a chemical composition capable of adopting a structure essentially equivalent to the pharmacophoric pattern of a section of the capsular polysaccharide of *Neisseria meningitidis* group B, optionally together with a pharmaceutically acceptable carrier or excipient for use as an anti-group B capsular polysaccharide immunogen.

2. Peptides which include an amino acid sequence capable of adopting a structure having an pharmacophoric pattern essentially equivalent to the pharmacophoric pattern of a section of the capsular polysaccharide of *Neisseria meningitidis* group B.

3. Antigenic peptide ligands which cross react with antibodies against the capsular polysaccharide of group B meningococci (CPS-B) which peptides include 1 or more copies of a motif which consists of a ring aromatic moiety, a spacer moiety, and a moiety having negative charge.

4. An antigenic peptide according to claim 3 having two or more of said motifs.

5. An antigenic peptide according to claim 4 wherein said motifs repeat in an overlapping frame.

6 An antigenic peptide which includes an amino acid sequence and structure essentially equivalent to the pharmacophore defined as follows:

the pharmacophore includes at least 3 chemical features; a Ring Aromatic, a Negative Charge and a Hydrogen Bond Acceptor or Donor feature; these features being further defined as follows:

(1A) the hydrogen bond acceptor feature matches the following atom types or groups of atoms which are surface accessible;

- sp or sp^2 nitrogens that have a lone pair and a charge less than or equal to zero
- sp^3 oxygens or sulphurs that have a lone pair and charge less than or equal to zero
- non-basic amines that have a lone pair;

(1B) the hydrogen bond donor feature has the same chemical characteristics as the hydrogen bond acceptor except that it also includes basic nitrogen (there is no exclusion of electron-deficient pyridines and imidazoles);

this feature matches the following atom types or groups of atoms;

- non-acidic hydroxyls
- thiols
- acetylenic hydrogens
- NH moieties (except tetrazoles and trifluoromethyl sulfonamide hydrogens);

(2) the negative charge feature is defined as a negative charge not adjacent to a positive charge; and

(3) the ring aromatic feature is defined as an aromatic moiety which may be replaced by amino acid residues having hydrophobic character;

this feature matches the following group;

methionine, alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and tyrosine; and

(ia) the hydrogen bond acceptor is represented by a vector function consisting of two spheres;

the smaller sphere (at least 1.6Angstroms radius up to 2.6 Angstroms) defines the centroid of the hydrogen bond acceptor on the ligand while the large sphere (at least 2.2Angstroms radius up to 2.6 Angstroms) defines the projected point of the hydrogen bond acceptor from the receptor;

these two spheres are at least 3.0Angstroms apart;

(ib) the hydrogen bond donor is represented by a two sphere vector function as (ia) above;

(ii) the negative charge is represented by a sphere at least 1.6Angstroms radius (up to 2.6 Angstroms); and

(iii) the ring aromatic is represented as two equal size spheres (at least 1.6Angstroms radius up to 2.0 Angstroms) whose centroids are 3.1Angstroms apart;

one sphere corresponds to the position of an aromatic ring moiety and the other to the projected point of the electron pi stacking of the aromatic ring system;

and wherein the tolerances on all distances between these features is +/- 0.5 Angstroms and the geometric angles +/- 20 Degrees and said distances and angles are shown in figure 1 or figure 2.

7. An antigenic peptide according to claim 6 having an additional fourth chemical feature which is a hydrogen bond donor/acceptor.

8. Antigenic peptides according to any one of claims 3 to 7 of 3 to 25 amino acids of the general formula:

X-B-C-D-Y

wherein

X is any group of amino acids of length P;

D is any hydrophobic amino acid group;

C is any amino acid;

B is any negative residue;

Y is any group of amino acids of length Q; and

P and Q may be zero and P + Q is less than or equal to 22.

9. A peptide according to any one of claims 3 to 8 having 6 to 20 amino acids.

10. A peptide according to any one of claims 3 to 9 having 9 to 15 amino acids.

11. A peptide according to claim 8 or 9 wherein P + Q is less than or equal to 17.

12. A peptide according to any one of claims 8 to 11 wherein P + Q is less than or equal to 8.

13. A peptide according to any one of claims 8 to 12 wherein X contains His.

14. A peptide according to any one of claims 8 to 13 wherein D is Tyr.

15. A peptide according to any one of claims 8 to 14 wherein C is an uncharged amino acid.

16. A peptide according to any one of claims 8 to 15 wherein C is Ser or Thr.

17. A peptide according to any one of claims 8 to 16 wherein C is Thr.

18. A peptide according to any one of claims 8 to 17 wherein B is an acidic residue.

19. A peptide according to any one of claims 8 to 18 wherein B is Asp or Glu.

20. A peptide according to any one of claims 8 to 19 wherein B is Glu.

21. A peptide according to any one of claims 8 to 20 wherein Y contains His.

22. A peptide according to any one of claims 8 to 21 having the general formula

X(containing a His)-Tyr-Thr-Glu-Y(containing a His).

23. Antigenic peptides of 5 to 25 amino acids of general formula:

X-ABCDE-Y

wherein

A is any amino acid;

E is any amino acid;

X is any group of amino acids of length P;

Y is any group of amino acids of length Q; and

P & Q may be zero and P + Q is less than or equal to 20 and B,C,D are as defined in claim 8.

24. A peptide according to claim 23 wherein the peptide is 6 to 20 amino acids.

25. A peptide according to claim 23 or 24 wherein the peptide is 9 to 15 amino acids.

26. A peptide according to any one of claims 23 to 25 wherein B is Asp or Glu.

27. A peptide according to any one of claims 23 to 26 wherein B is Asp.

28. A peptide according to any one of claims 23 to 27 wherein A is Met.

29. A peptide according to any one of claims 23 to 28 wherein E is His.

30. A peptide according to any one of claims 23 to 29 wherein P + Q is less than or equal to 15.

31. A peptide according to any one of claims 23 to 30 wherein P + Q is less than or equal to 10.

32. A peptide according to any one of claims 23 to 31 wherein the peptide has the general formula X-Met-Asp-Arg-Tyr-His-Y [SEQ ID NO:33].

33. A peptide according to any preceding claim which either alone or as a conjugate is immunogenic.

34. An immunogenic ligand which comprises a synthetic polypeptide selected from compounds of sequence SEQ ID NO:1:

TIPLWFDDEIEVMIY

and analogue and homologue derivatives thereof by virtue of one or more amino acid addition, deletion, substitution; together with terminal functional derivatives thereof.

35. An immunogenic ligand which comprises a synthetic polypeptide selected from compounds of sequence SEQ ID NO:2:

GDNFESYACVDTPCS

and analogue and homologue derivatives thereof by virtue of one or more amino acid addition, deletion, substitution; together with terminal functional derivatives thereof.

36. An immunogenic ligand which comprises a synthetic polypeptide selected from compounds of sequence SEQ ID NO: 38:

GECPTGSLLLDGLYC

and analogue and homologue derivatives thereof by virtue of one or more amino acid addition, deletion, substitution,; together with terminal functional derivatives thereof.

37. An immunogenic ligand according to any of claims 34 to 36 wherein the synthetic polypeptide is not more than 20 amino acids in length.

38. An immunogenic ligand according to any of claims 34 to 37 wherein the synthetic polypeptide is no less than 5 amino acids in length.

39. An antigenic peptide which comprises one or more of the trimeric motif sequences selected from the group which comprises:

NFD; FDD; DEI; and EVM

or palandromes thereof.

40. An antigenic peptide which comprises one or more of the trimeric motif sequences selected from the group which comprises:

DNF; ESY; and DTP

41. An antigenic peptide which comprises one or more of the trimeric motif sequences selected from the group which comprises:

ECP; LLD; and DGL

or palandromes thereof.

42. An antigenic peptide according to any one of claims 39 to 41 which comprises two or more trimeric motif sequences.

43. An antigenic peptide according to claim 42 wherein said trimeric motif sequences repeat in an overlapping frame.

44. An immunogenic ligand according to any preceding claim which is the pentadecapeptide of SEQ ID NO:1

TIPLWFDDEIEVMIY

or the pentadecapeptide of SEQ ID NO: 2

GDNFESYACVDTPCS

or the pentadecapeptide of SEQ ID NO: 38

GECPTGSLLLDGLYC

and terminal functional derivatives thereof.

45. An immunogenic ligand according to any preceding claim which is the pentadecapeptide of SEQ ID NO:1

TIPLWFDDEIEVMIY

or the pentadecapeptide of SEQ ID NO: 2

GDNFESYACVDTPCS

or the pentadecapeptide of SEQ ID NO: 38

GECPTGSLLLDGLYC

with N- or C- terminal cysteine substitution.

46. An immunogenic ligand according to any preceding claim in which the synthetic polypeptide is conjugated to a carrier.

47. An immunogenic ligand according to claim 46 in which the carrier is a protein.

48. An immunogenic ligand according to claim 46 or 47 in which the carrier is covalently bonded to the synthetic polypeptide.

49. An immunogenic ligand according to any of claims 46, 47 or 48 in which the synthetic polypeptide is linked to a carrier selected from KLH, Diphtheria toxoid, and Tetanus toxoid.

50. An immunogenic ligand according to any one of claims 46 to 49 in which the synthetic polypeptide is linked to KLH by glutaraldehyde.

51. An immunogenic ligand according to any one of claims 46 to 49 in which the synthetic polypeptide is linked to KLH via the heterobifunctional linker MBS.

52. An immunogenic ligand which comprises a bacteriophage which encodes and is capable of expressing a synthetic polypeptide as defined in any of claims 34 to 38.

53. An antibody produced by an immune response to an immunogenic ligand according to any preceding claim, which antibody specifically recognises and binds the synthetic polypeptide of the ligand and also the group-B meningococcal Capsular Polysaccharide (CPS-B).

54. An antibody according to claim 53 which is monoclonal.

55. An antibody according to claim 53 or 54 which is humanised.

56. A process for producing ligand according to any one of claims 2 to 45 which comprises synthesising a polypeptide as defined in any of claims 2 to 45 and optionally conjugating said synthetic polypeptide to a carrier.

57. A process for producing an antibody according to any of claims 53 to 55 which comprises producing an antigenic ligand by the process of claim 18 and subsequently administering said antigenic ligand in vivo

or in vitro so as to elicit an immune response resulting in production of said antibody

58. A process according to claim 57 including the further step of isolating and purifying said antibody.

59. A pharmaceutical composition containing as active ingredient at least one ligand according to any of claims 2 to 52, and optionally including an adjuvant or excipient.

60. A pharmaceutical composition containing as active ingredient at least one antibody according to any of claims 53 to 55, and optionally including an adjuvant or excipient.

61. A pharmaceutical composition according to claim 59 or 60 for use in the treatment of infection by group-B meningococcal bacteria.

62. A pharmaceutical composition according to claim 59 or 60 for use in prophylactic prevention of infection by group-B meningococcal bacteria.

63. A method of treatment of infection with group-B meningococcal bacteria, which comprises administering an effective amount of a ligand as defined in any of claims 2 to 52.

64. A method of treatment of infection with group-B meningococcal bacteria, which comprises administering an

effective amount of an antibody according to any of claims 53 to 55.

65. A method of treatment of infection with group-B meningococcal bacteria, which comprises administering an effective amount of a pharmaceutical composition according to claim 59 or 60.

66. A method of prophylaxis by prevention of infection with group-B meningococcal bacteria, which comprises administering an effective amount of a ligand according to any one of claims 2 to 52.

67. A method of prophylaxis by prevention of infection with group-B meningococcal bacteria, which comprises administering an effective amount of an antibody according to any of claims 53 to 55.

68. A method of prophylaxis by prevention of infection with group-B meningococcal bacteria, which comprises administering an effective amount of a pharmaceutical composition according to claim 59 or 60.

69. Use of a ligand according to claims 2 to 52 in the manufacture of a medicament for the treatment of infection with group-B meningococcal bacteria.

70. Use of an antibody according to claims 53 to 55 in the manufacture of a medicament for the treatment of infection with group-B meningococcal bacteria.

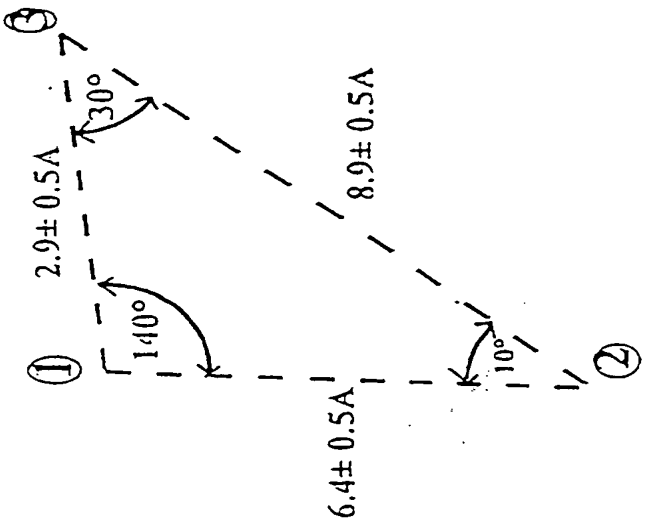
71. Use of a pharmaceutical composition according to claim 59 or 60 in the manufacture of a medicament for the treatment of infection with group-B meningococcal bacteria.

72. Use of a ligand according to claims 2 to 52 for the manufacture of a medicament for prophylactic prevention of infection with group-B meningococcal bacteria.

73. Use of an antibody according to claims 53 to 55 for the manufacture of a medicament for prophylactic prevention of infection with group-B meningococcal bacteria.

74. Use of a pharmaceutical composition according to claim 59 or 60 for the manufacture of a medicament for prophylactic prevention of infection with group-B meningococcal bacteria.

75. An immunogenic ligand, antibody (other than known antibody IgM^{Nov}) or pharmaceutical composition substantially as hereinbefore described.



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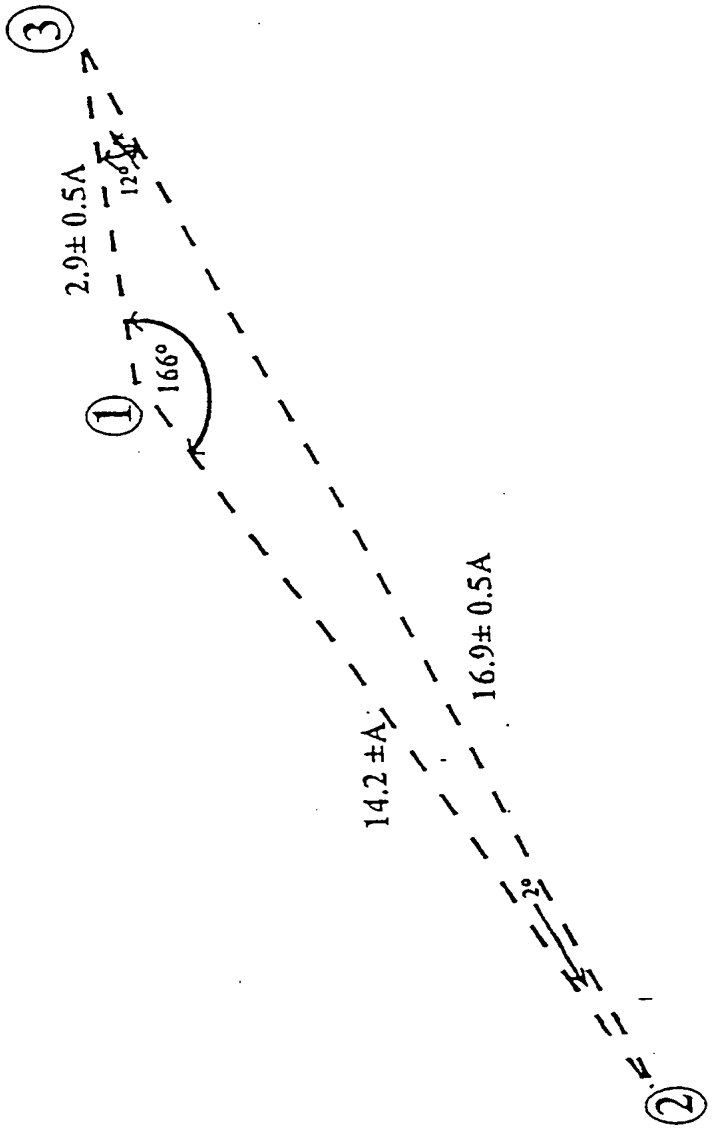

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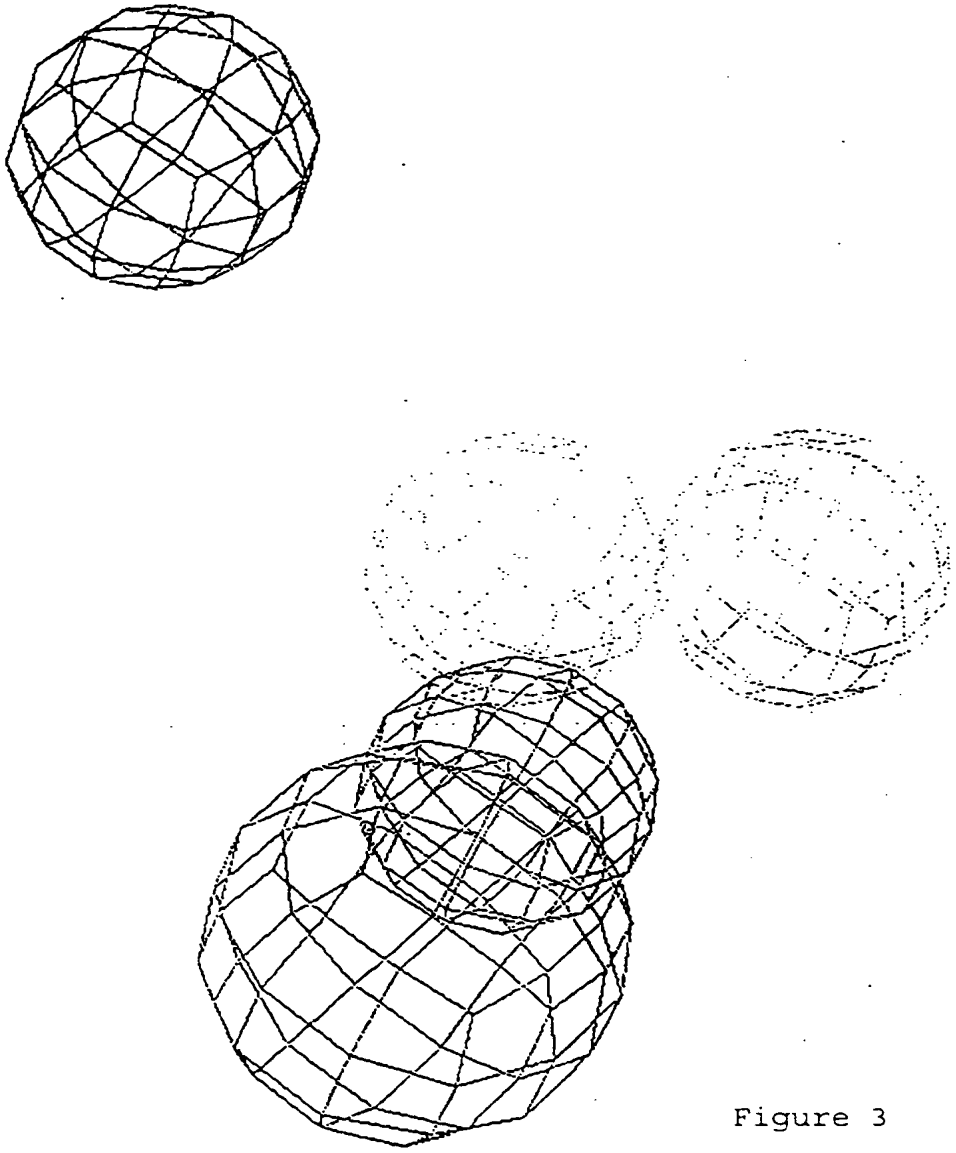

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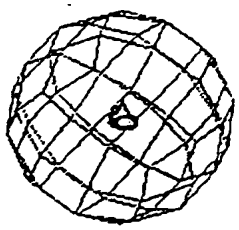

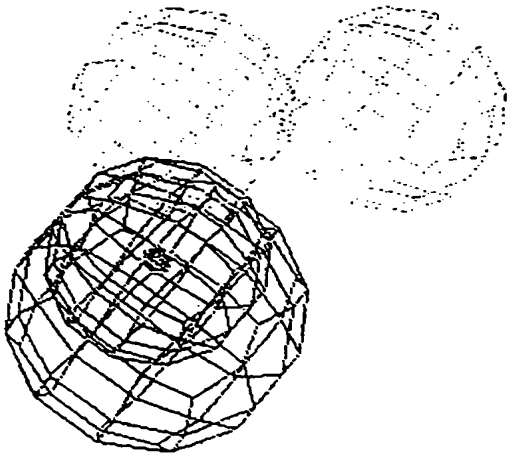

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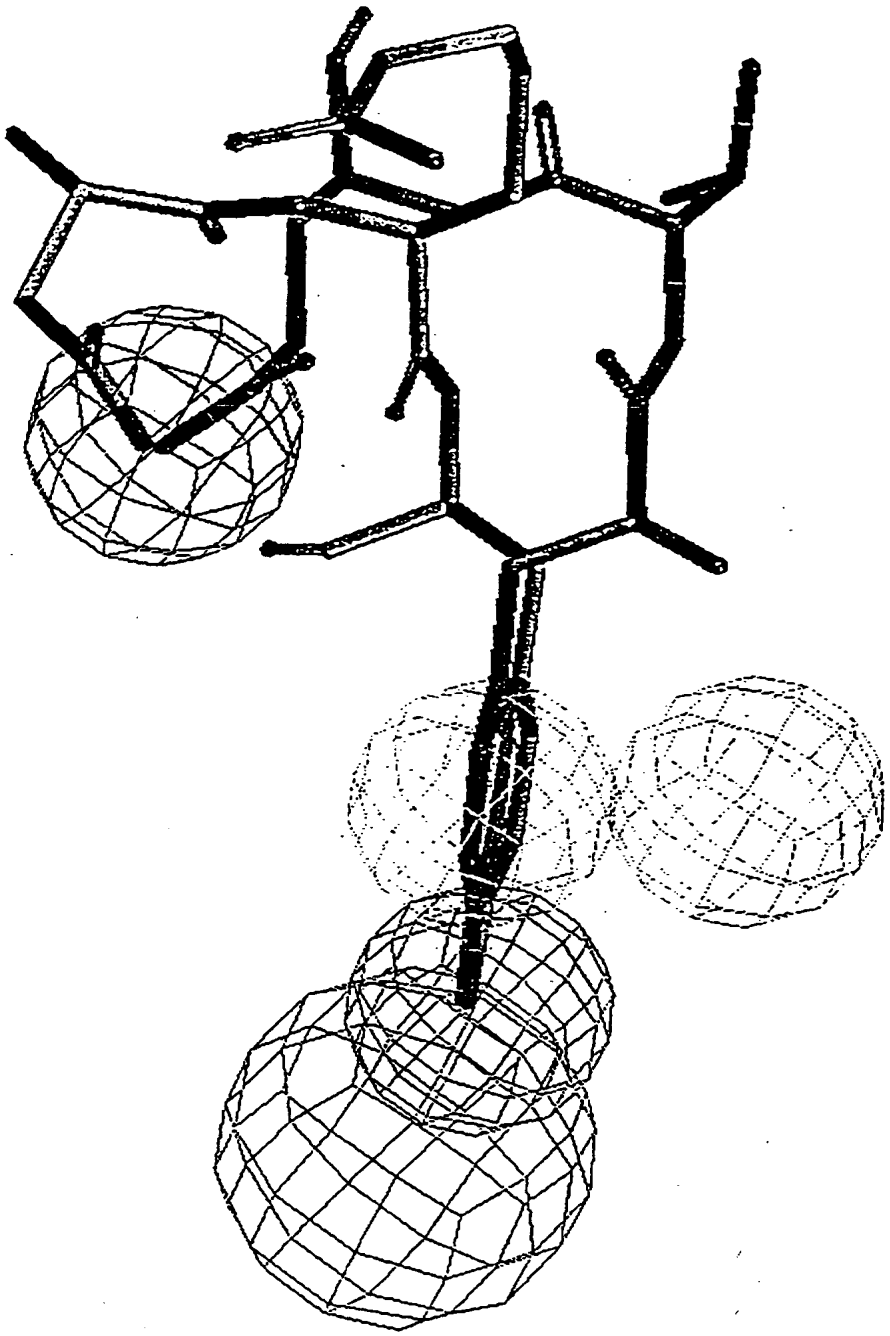

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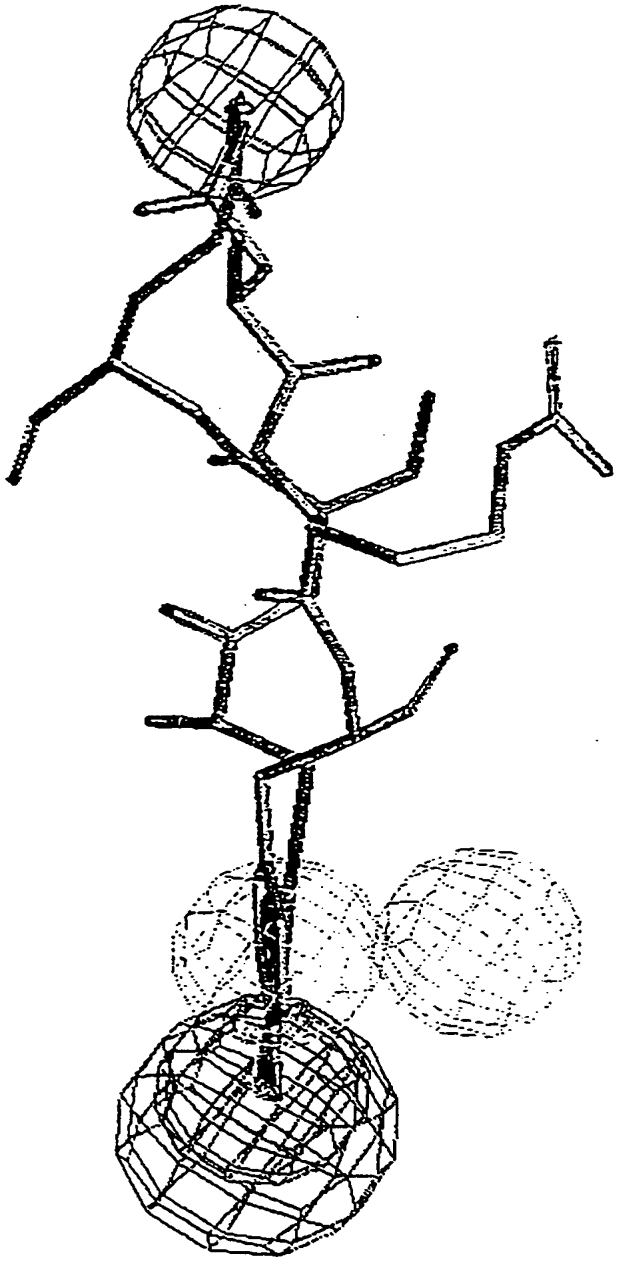

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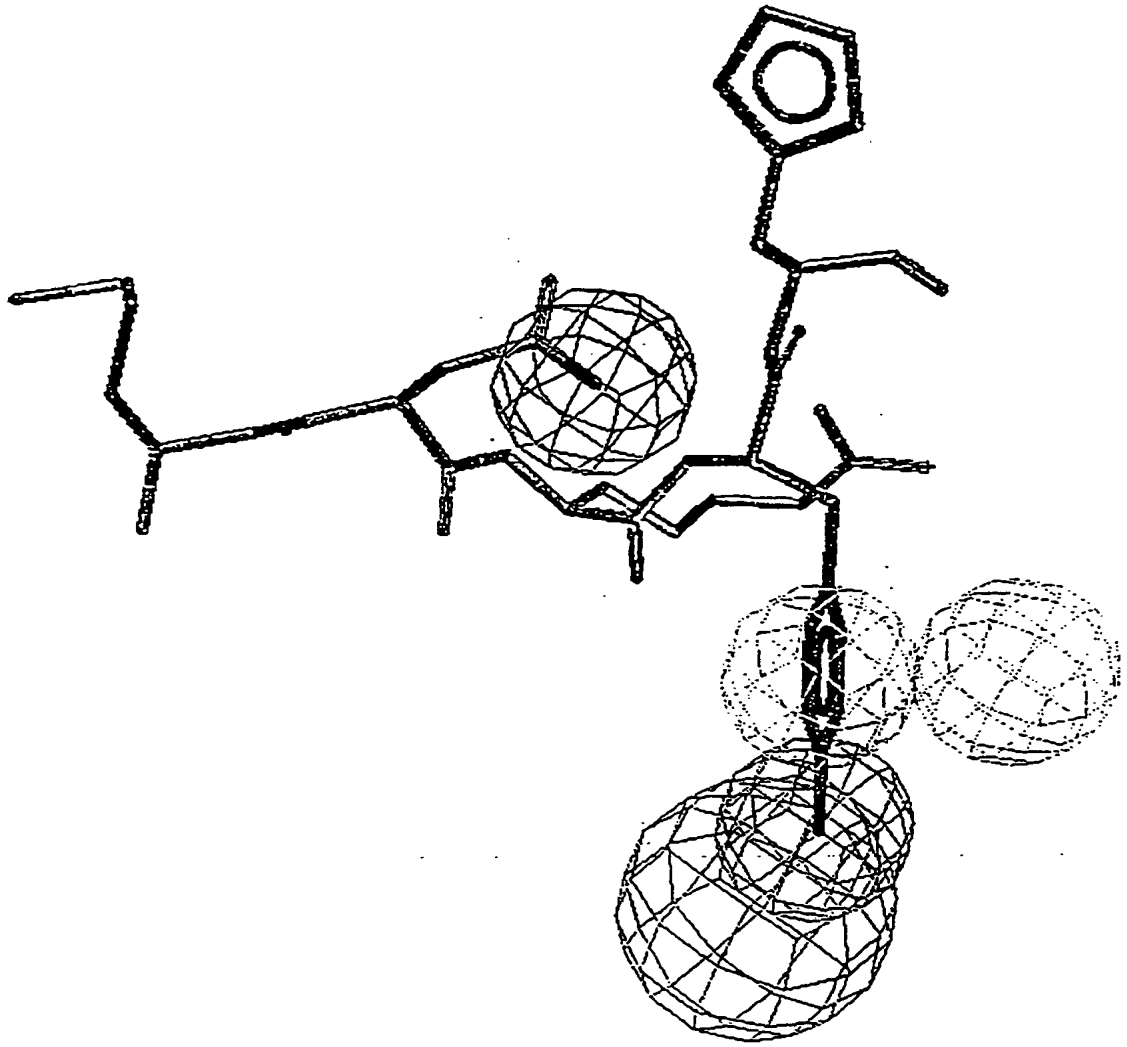

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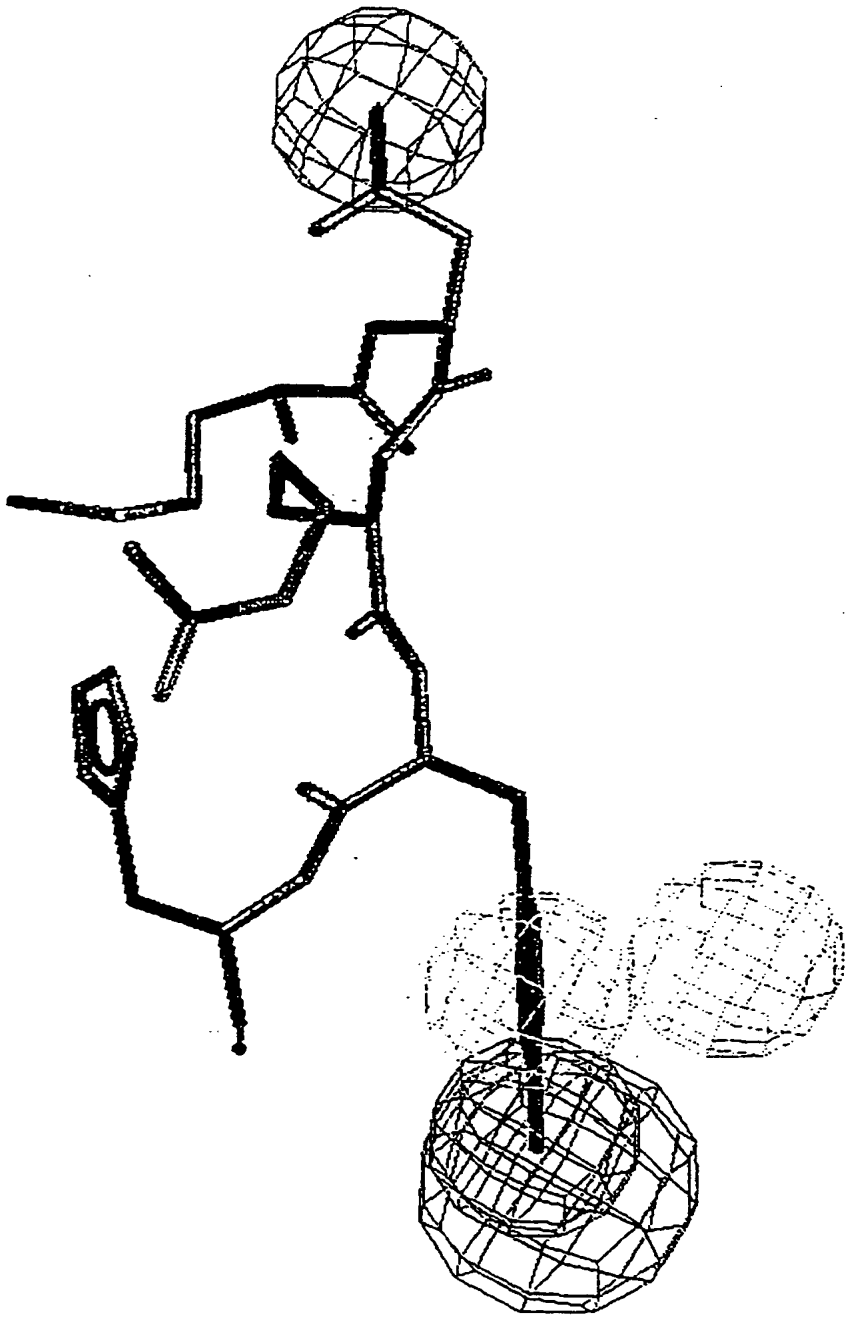

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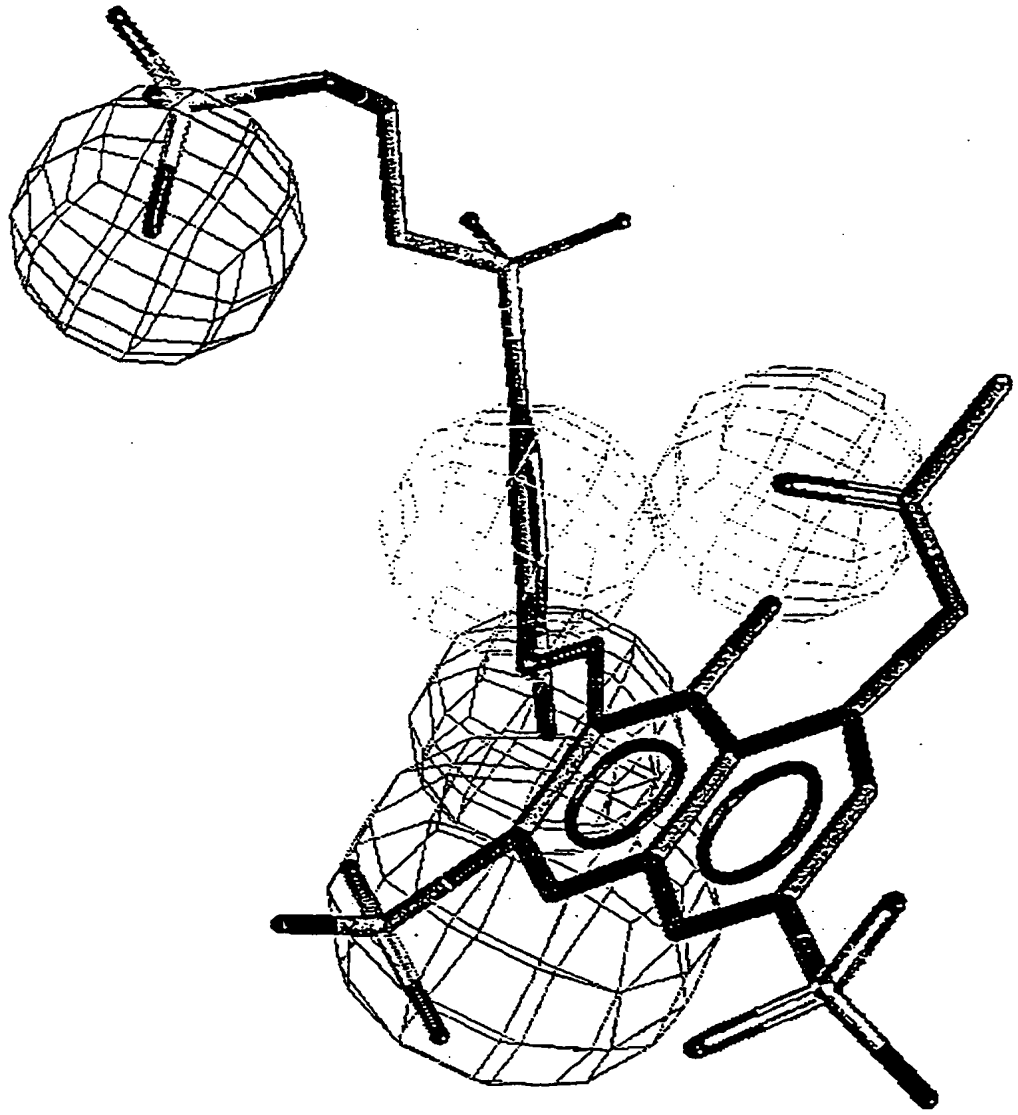

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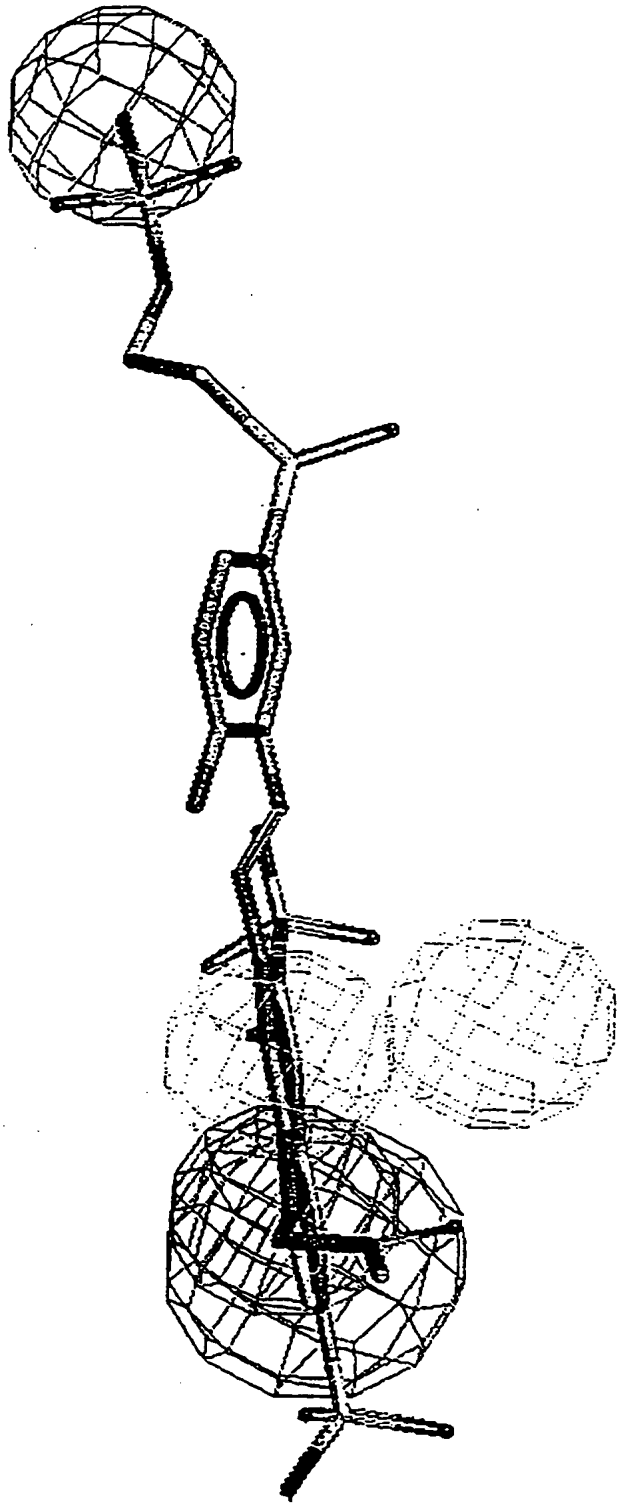

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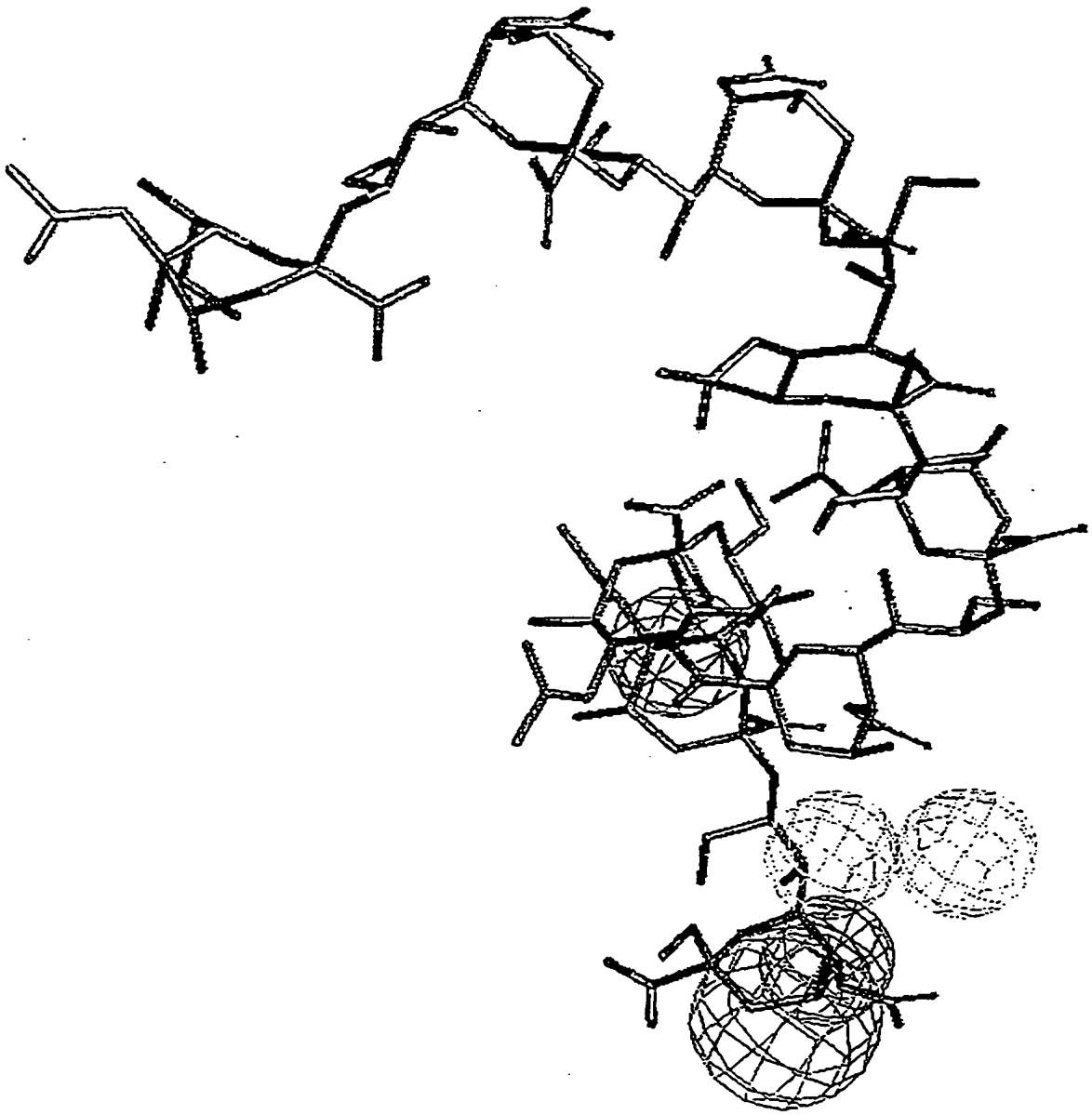

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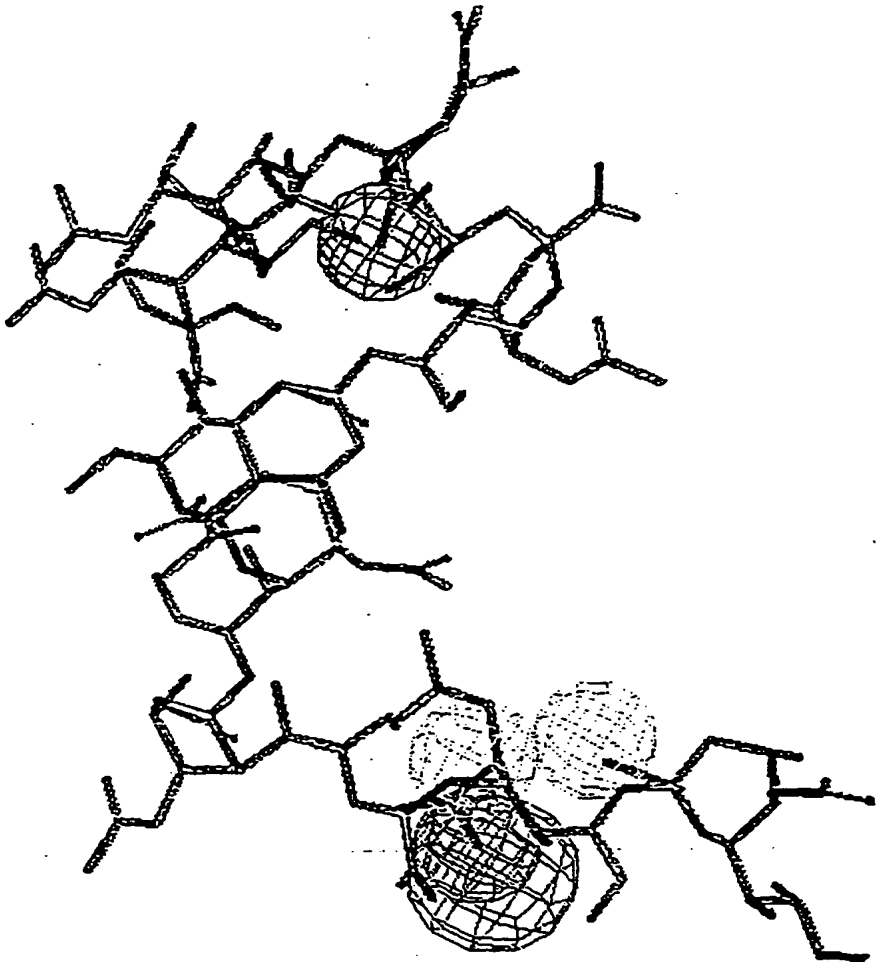

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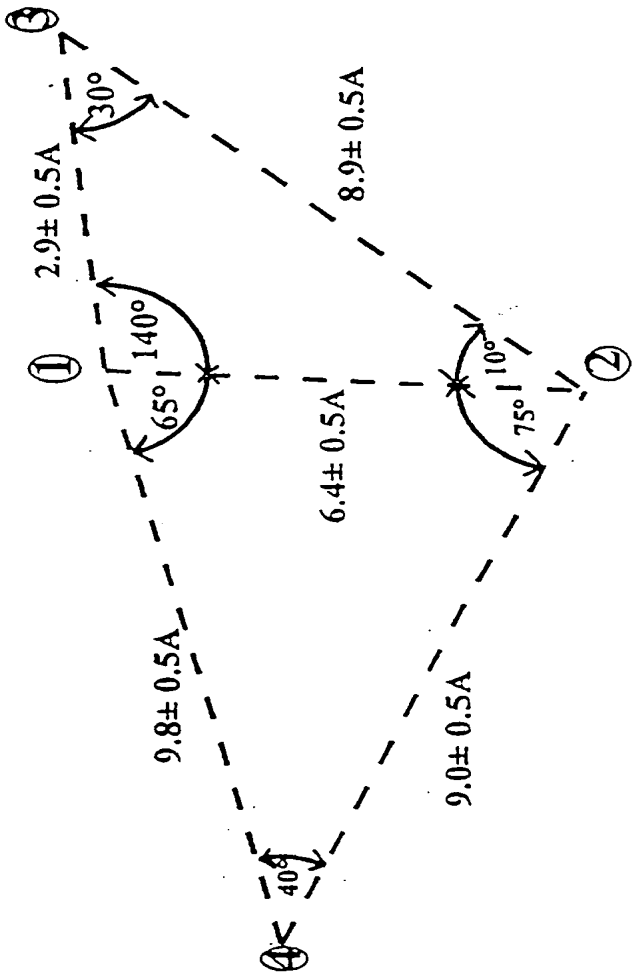

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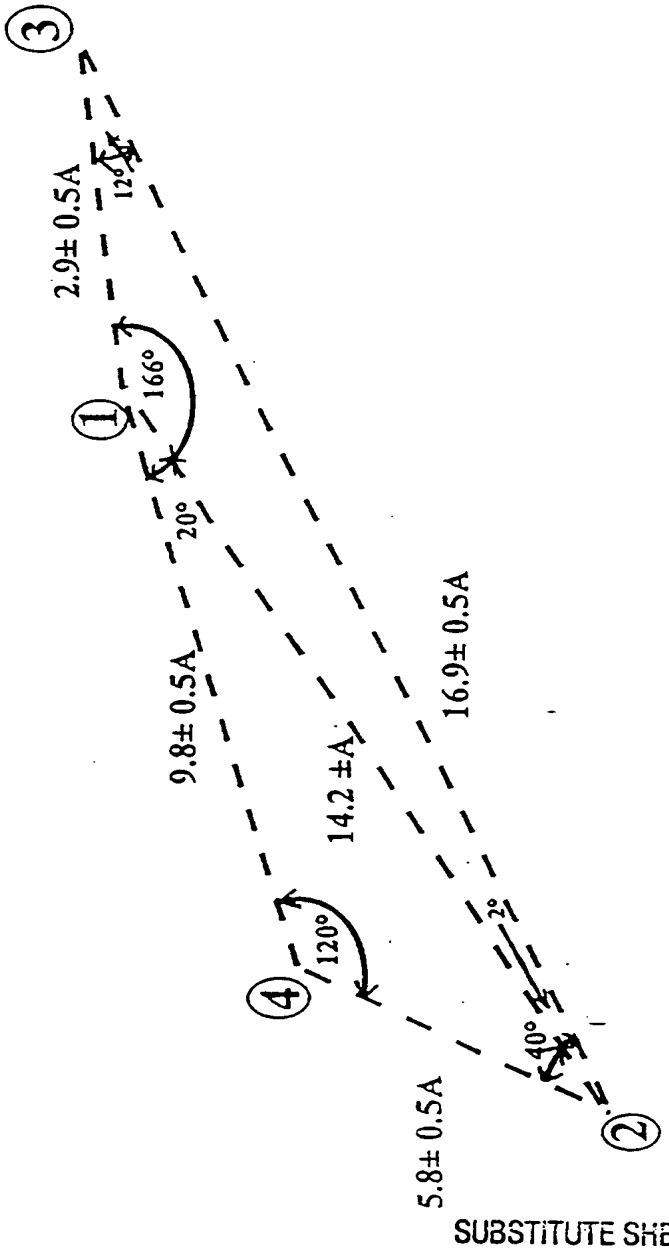

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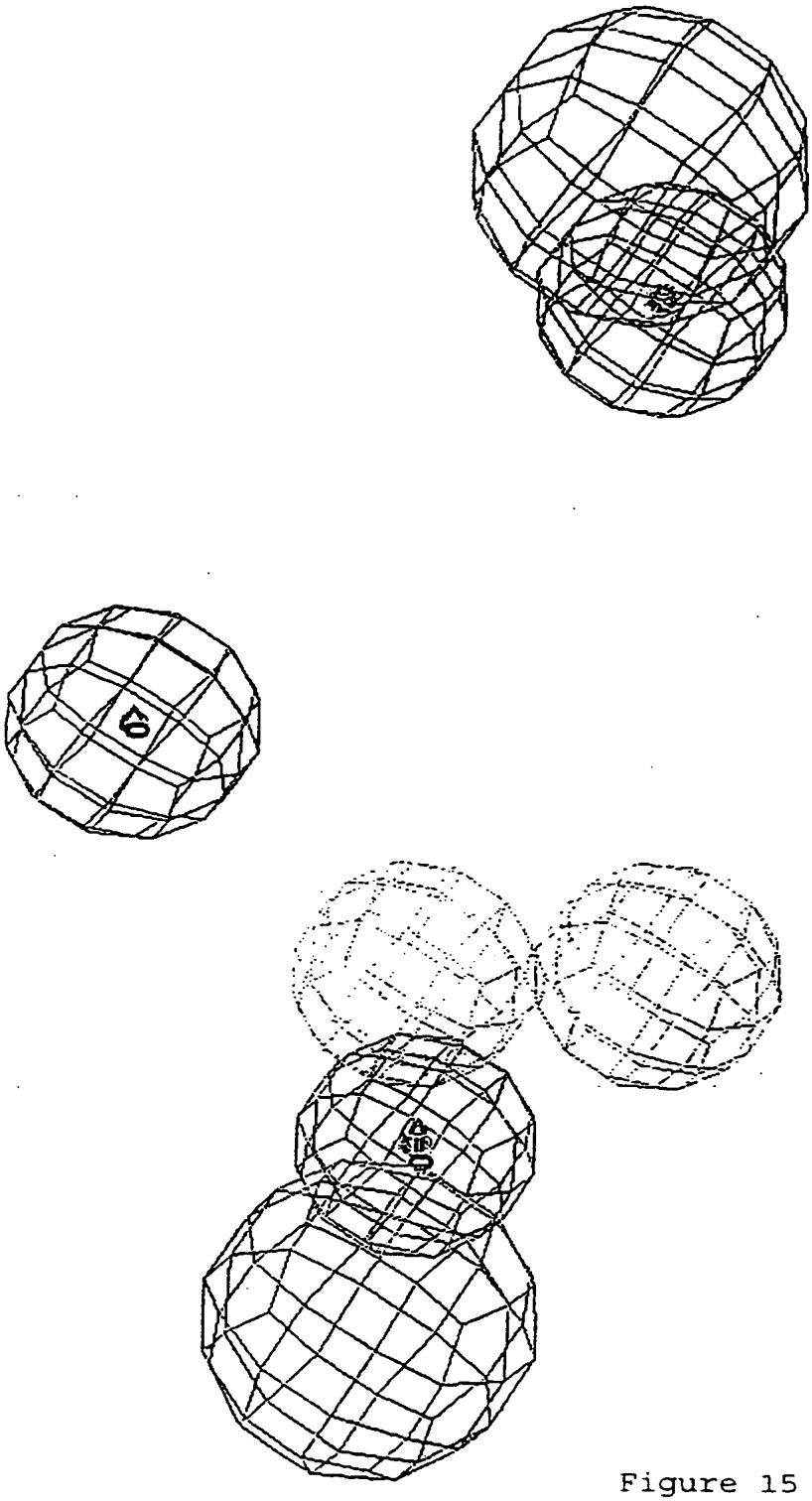

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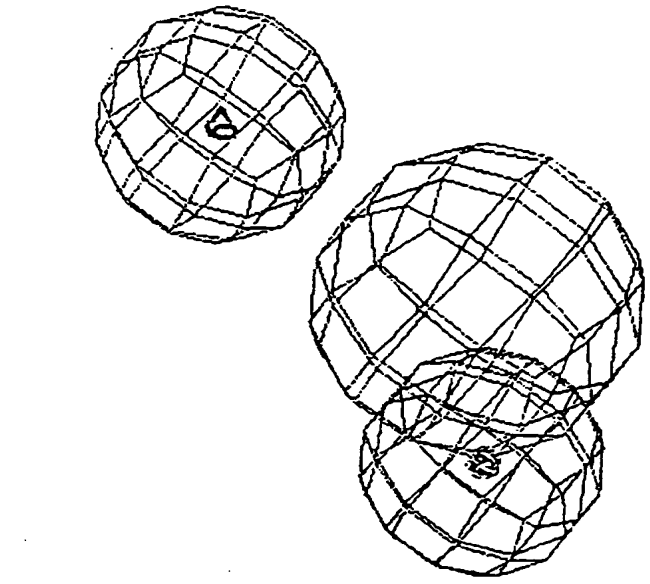

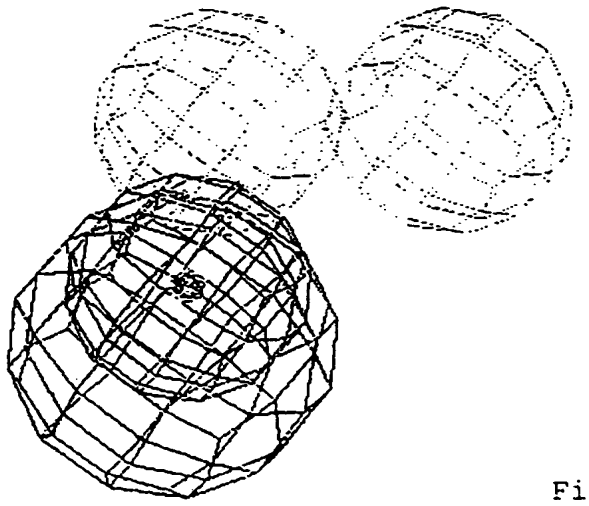

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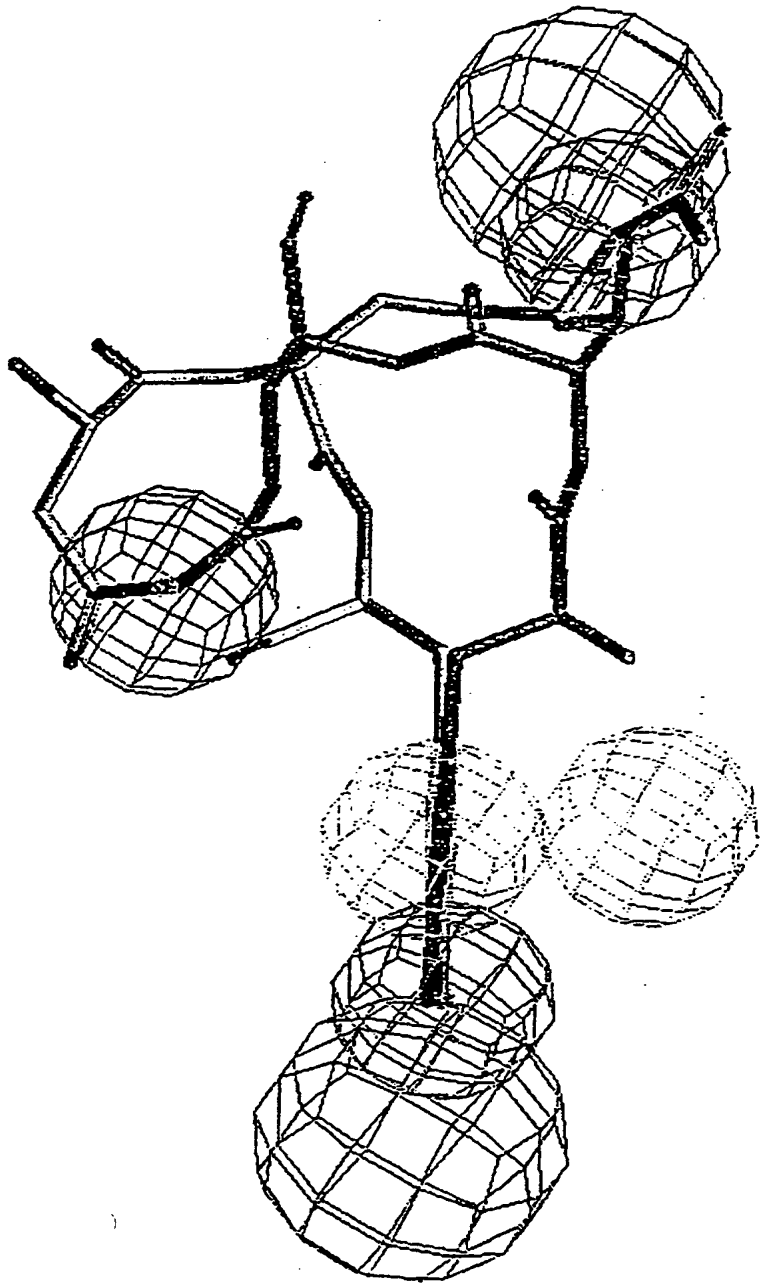

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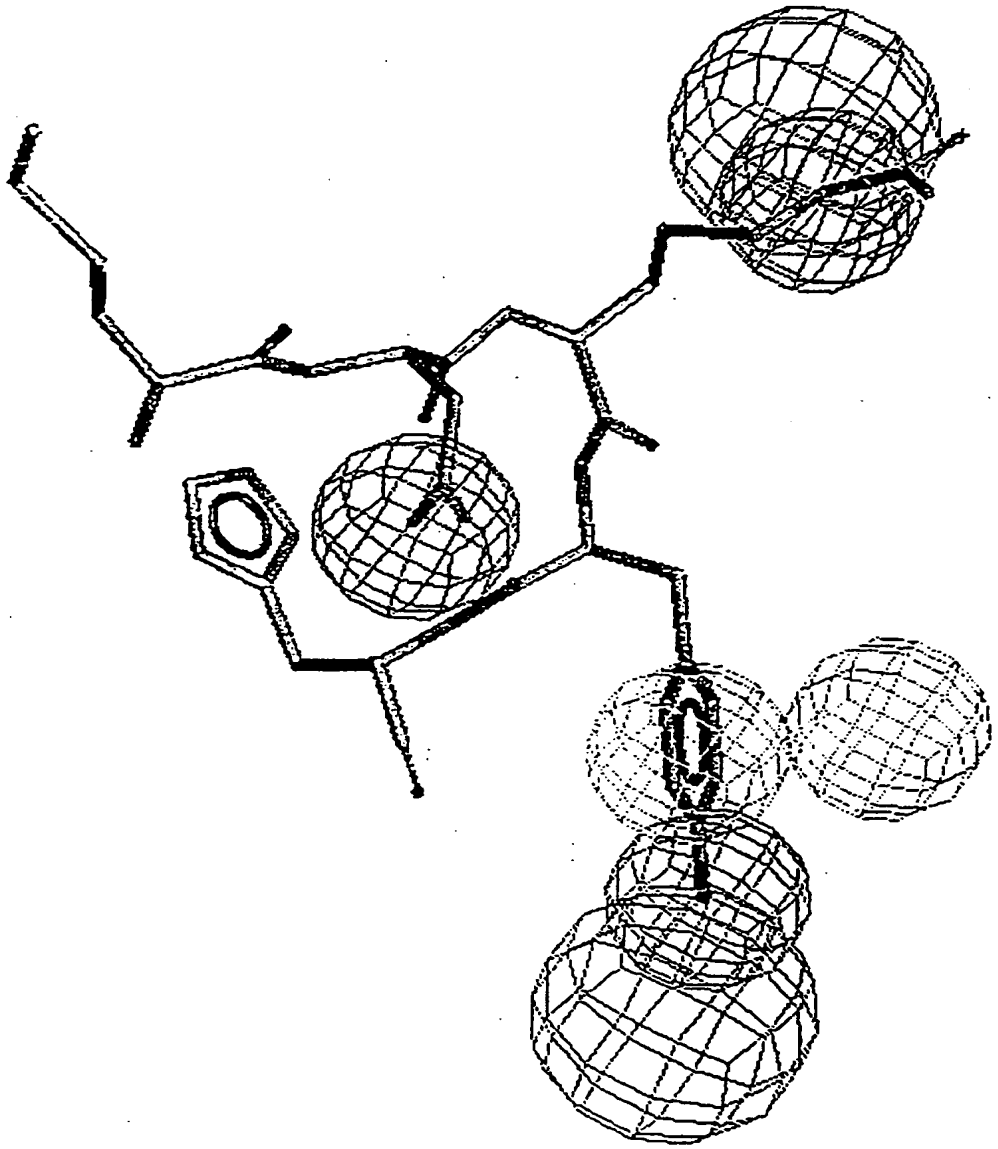

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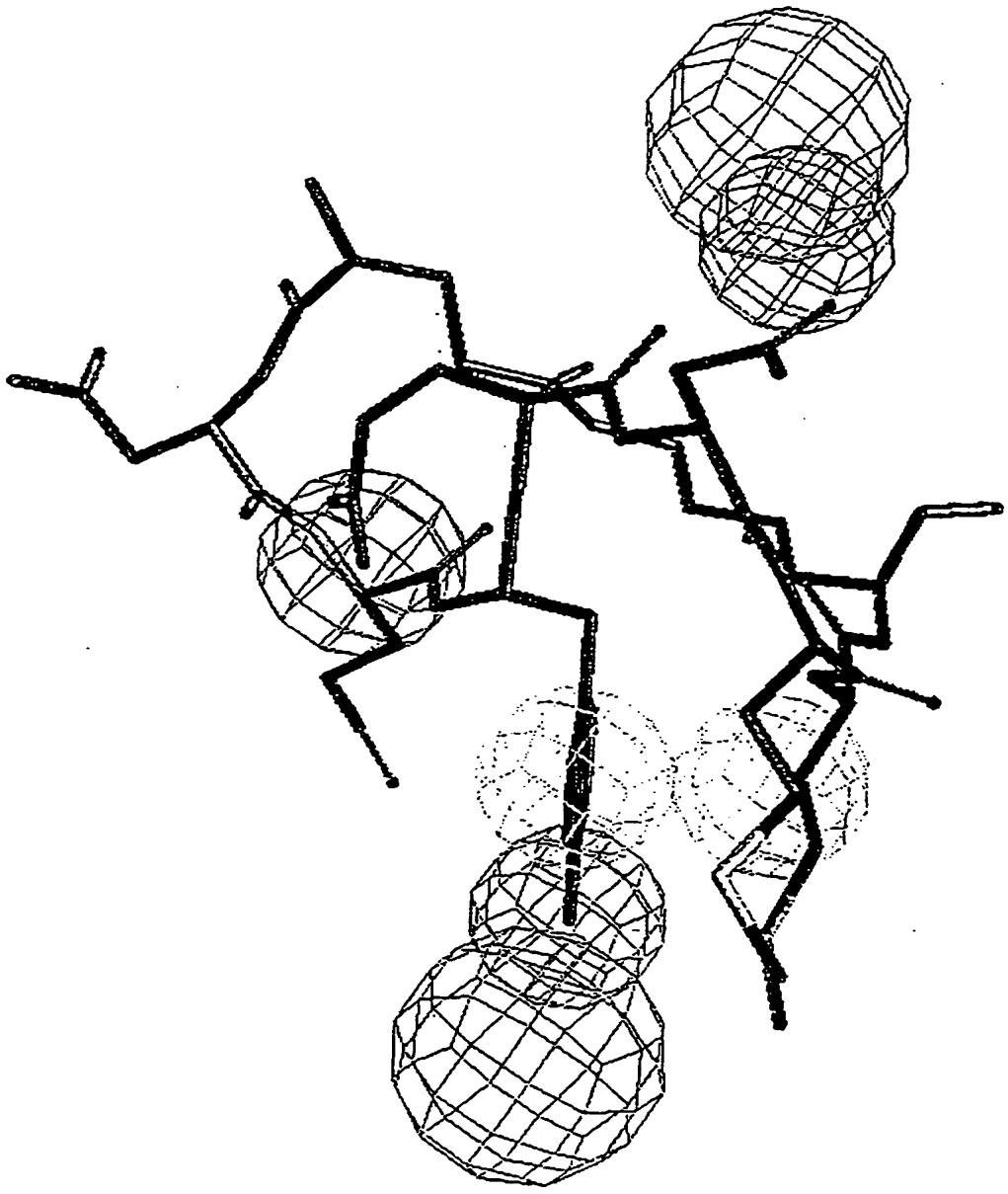

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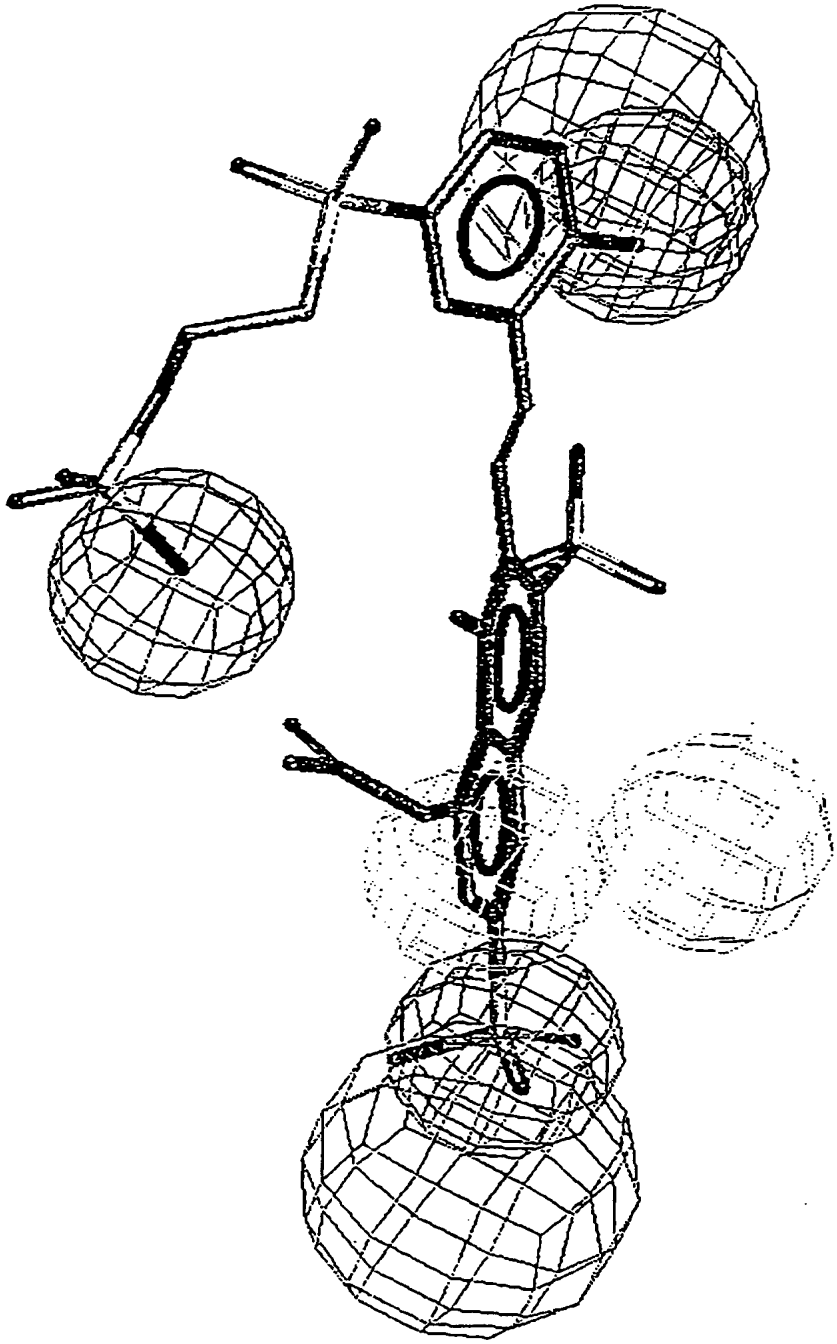

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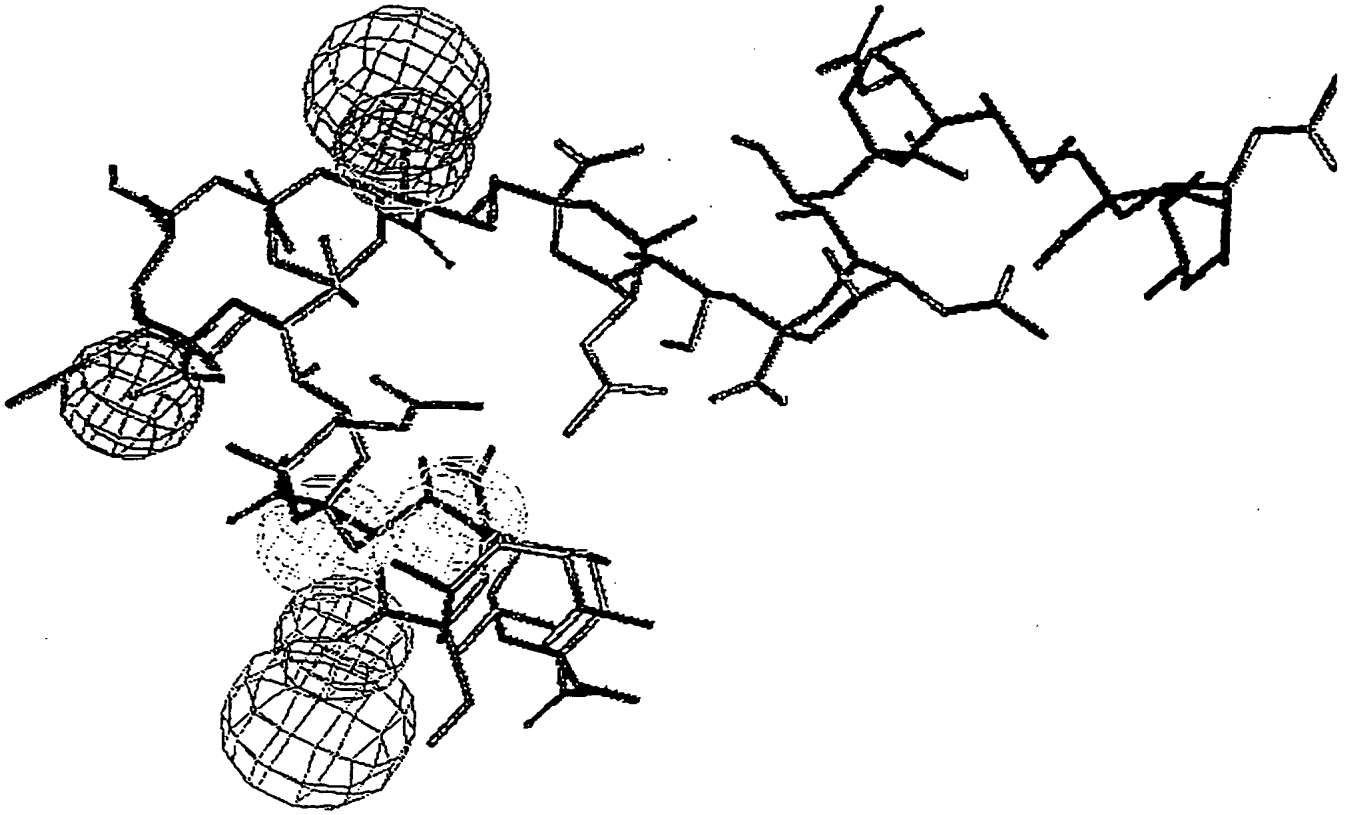

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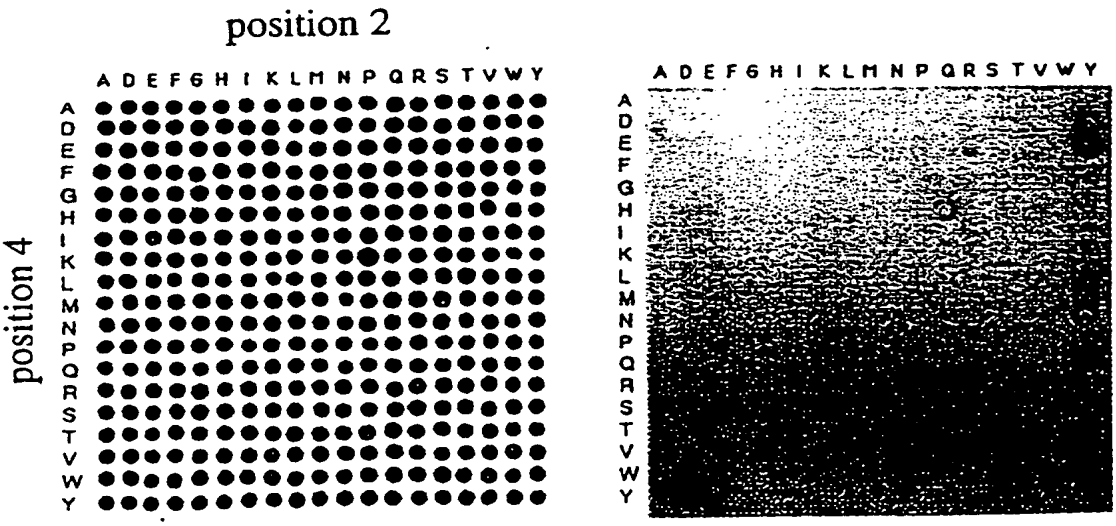

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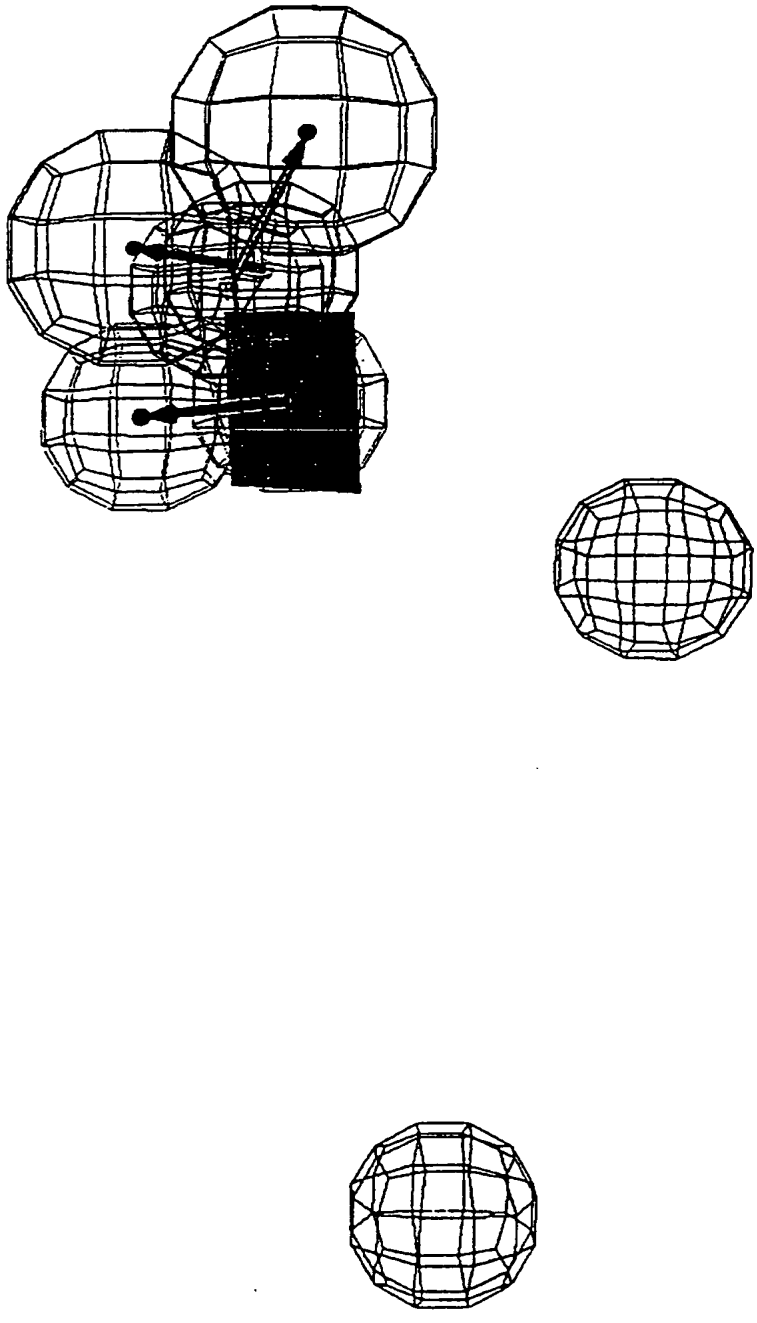

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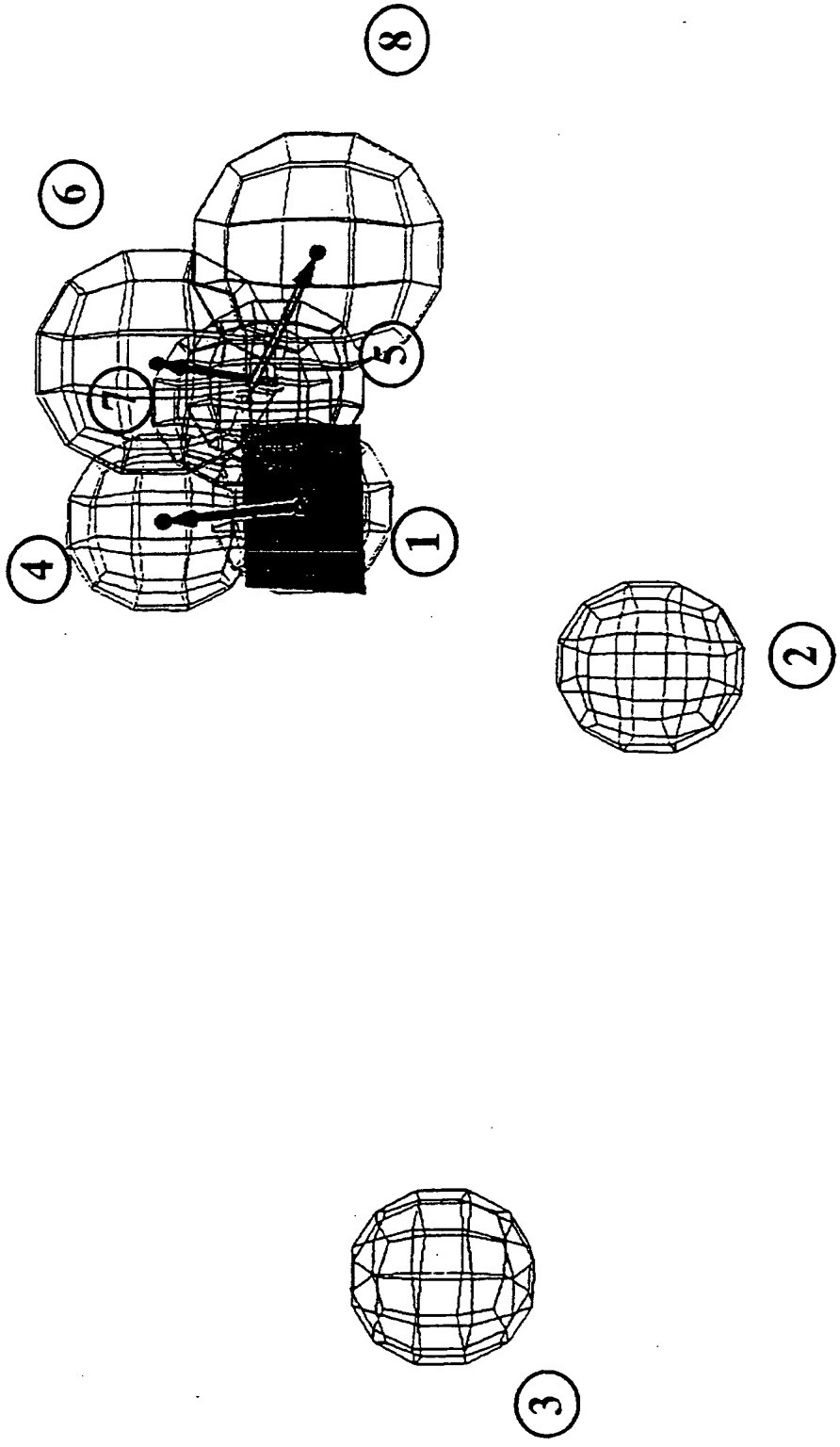

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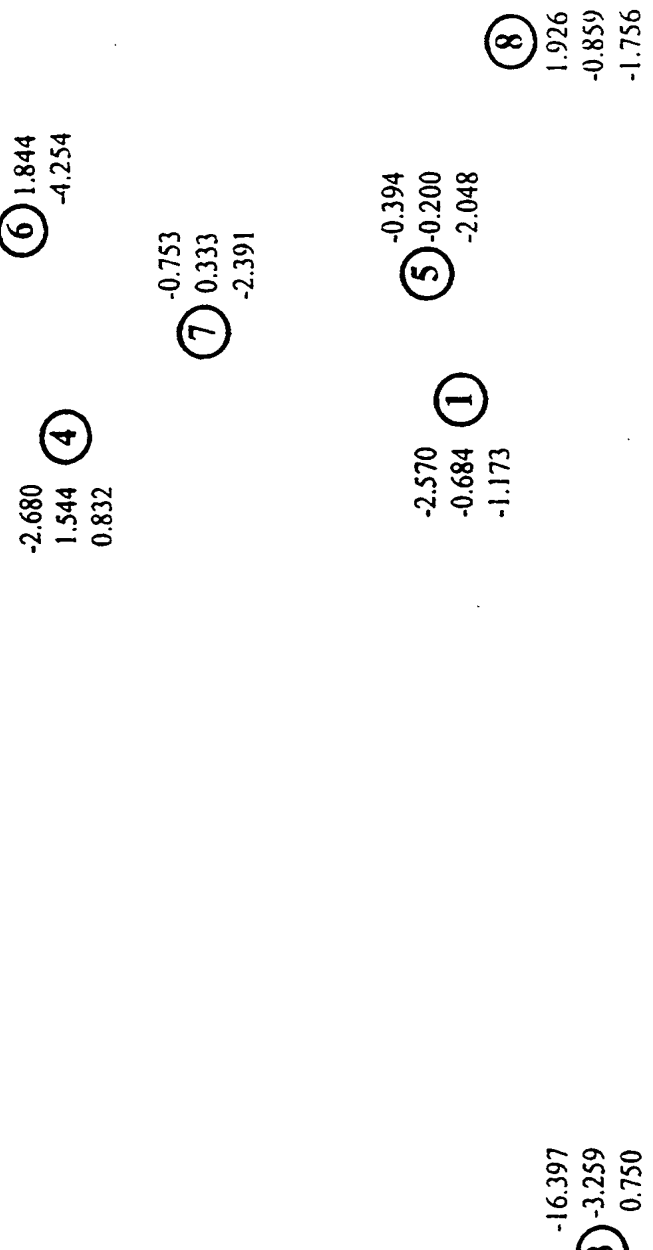

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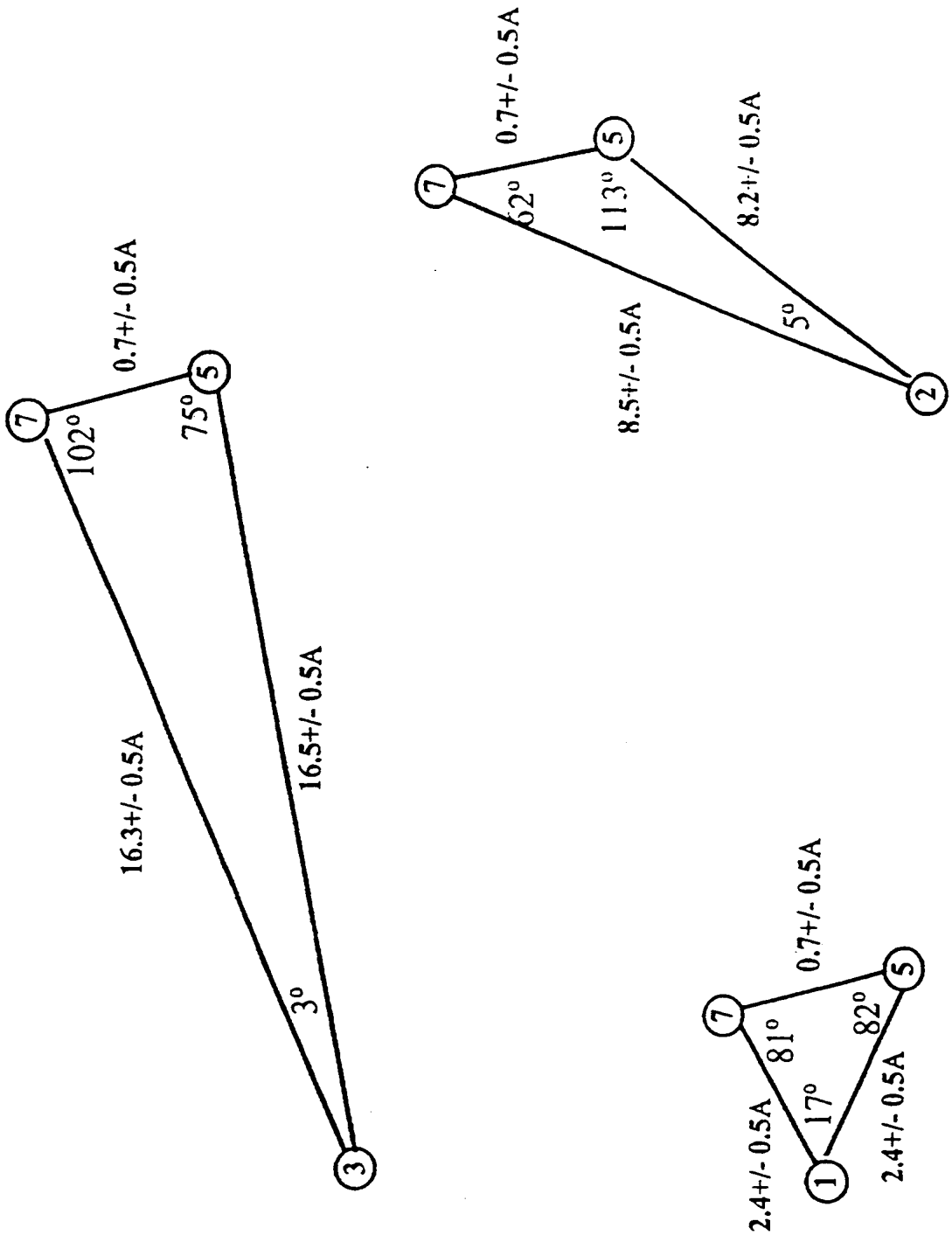

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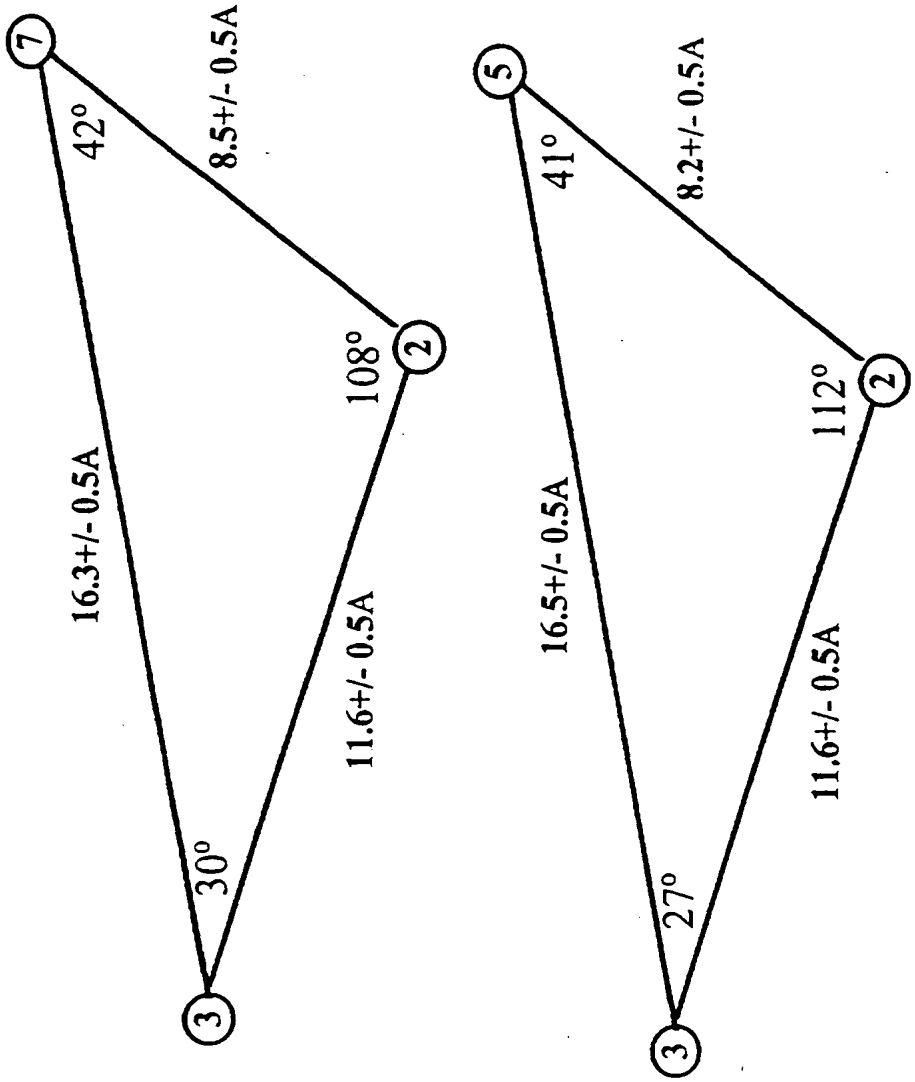

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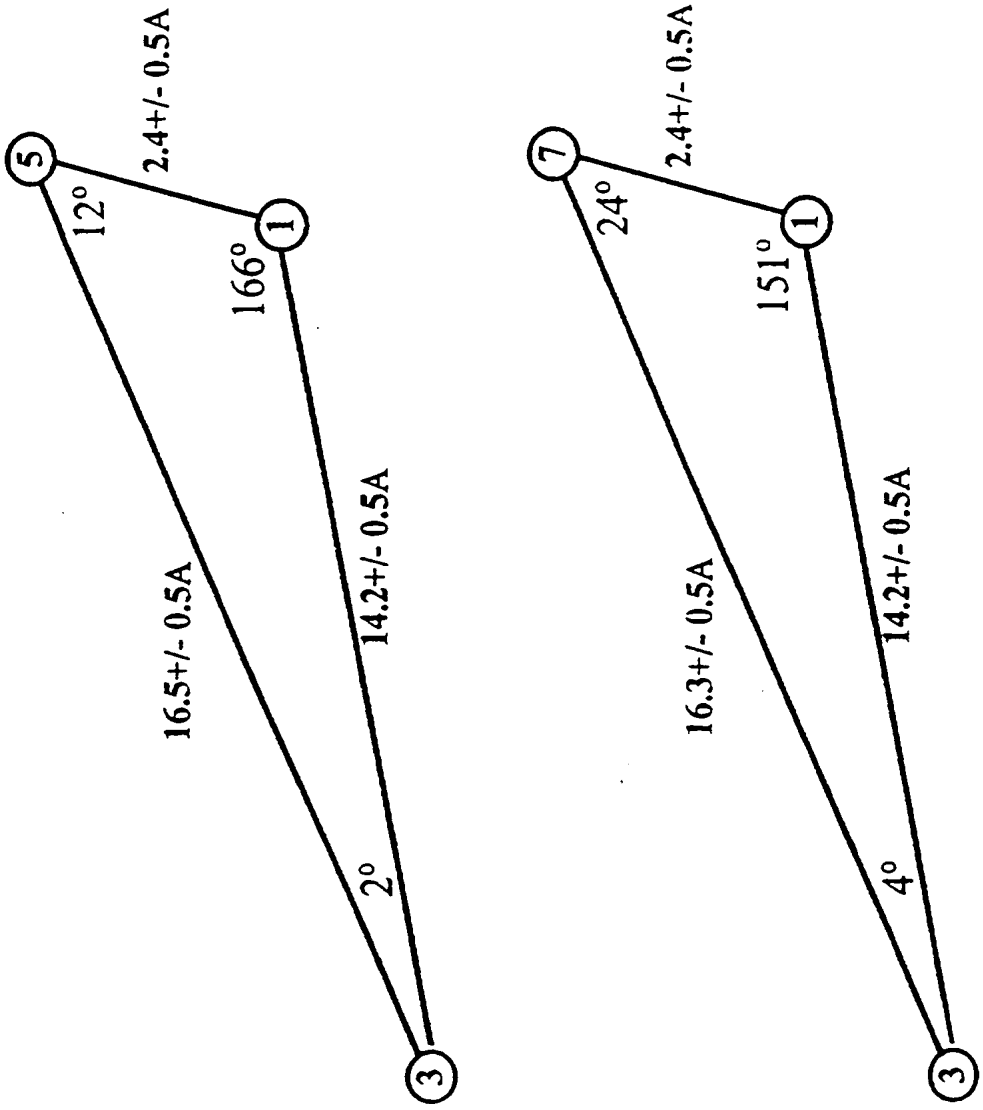

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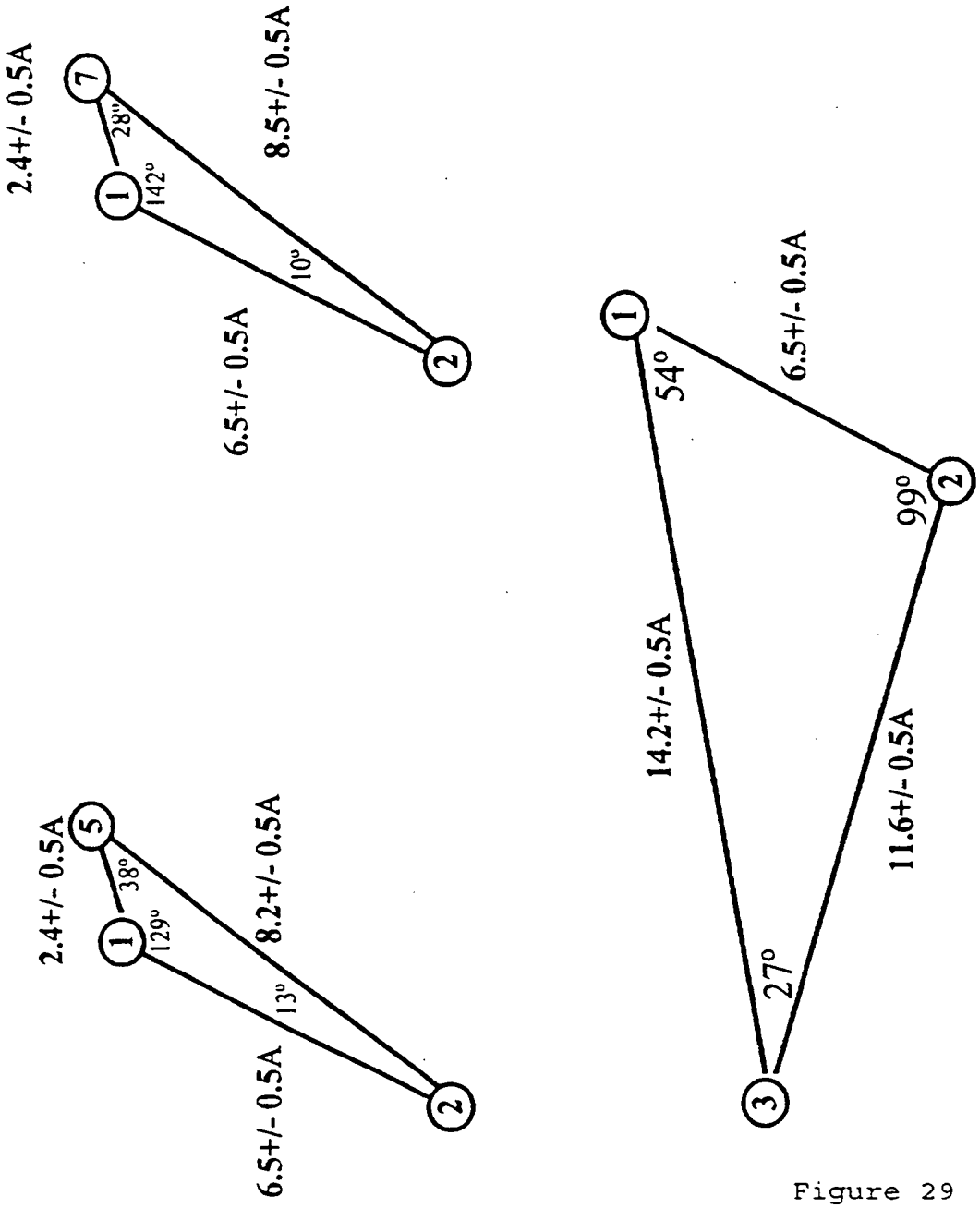

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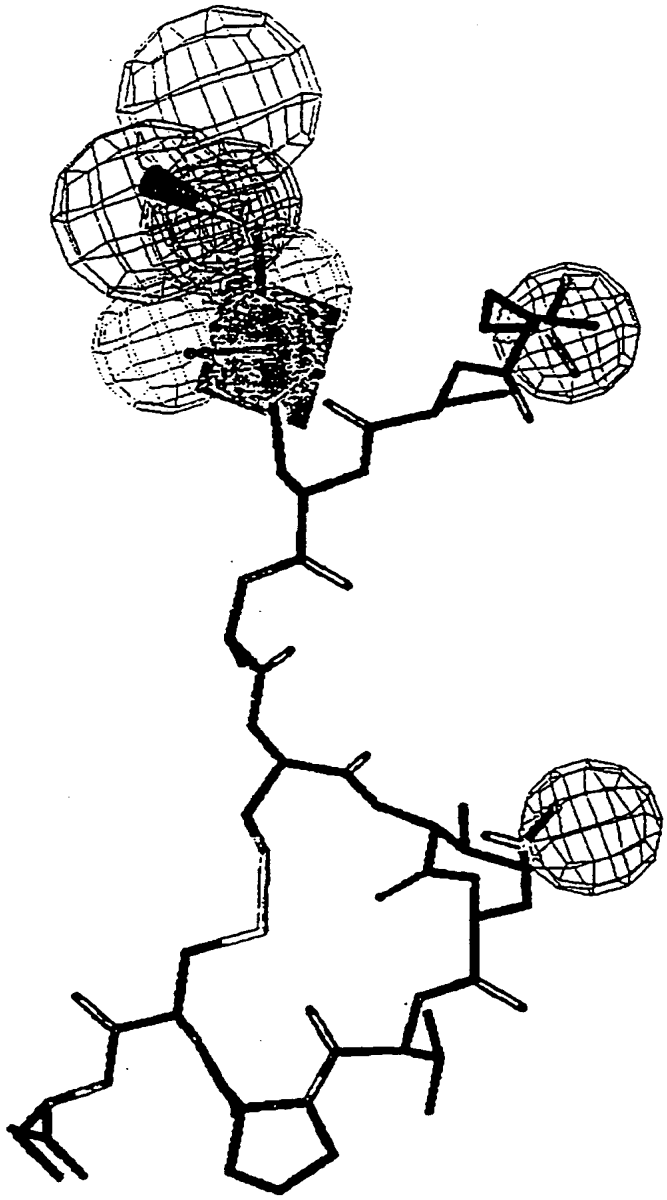

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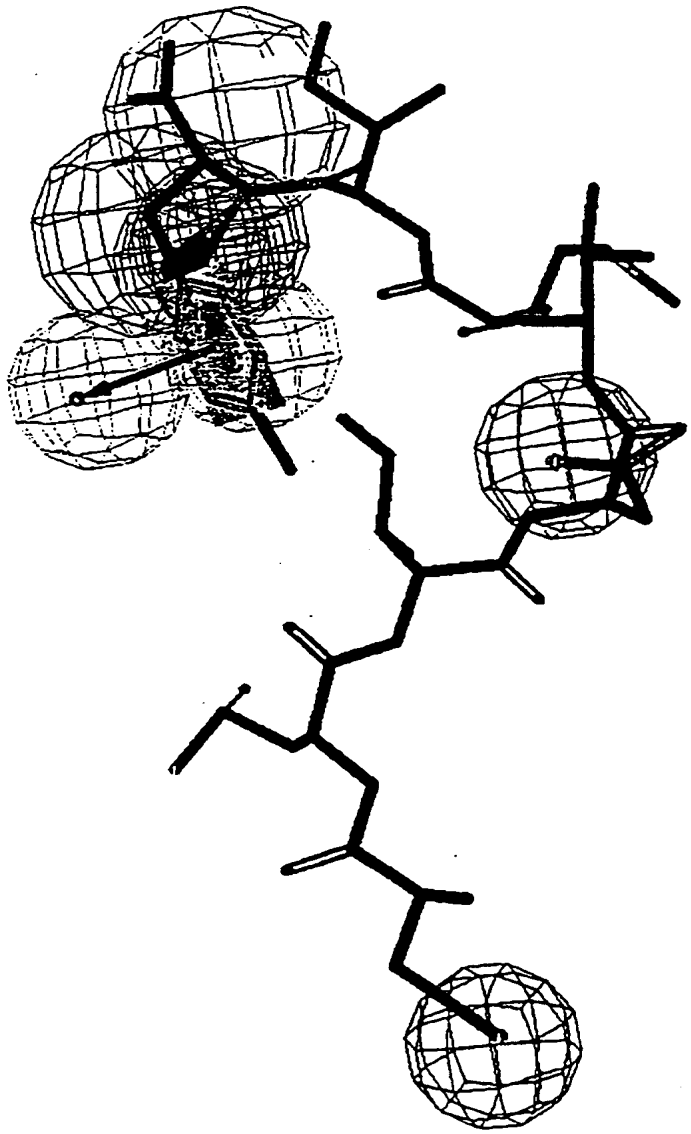

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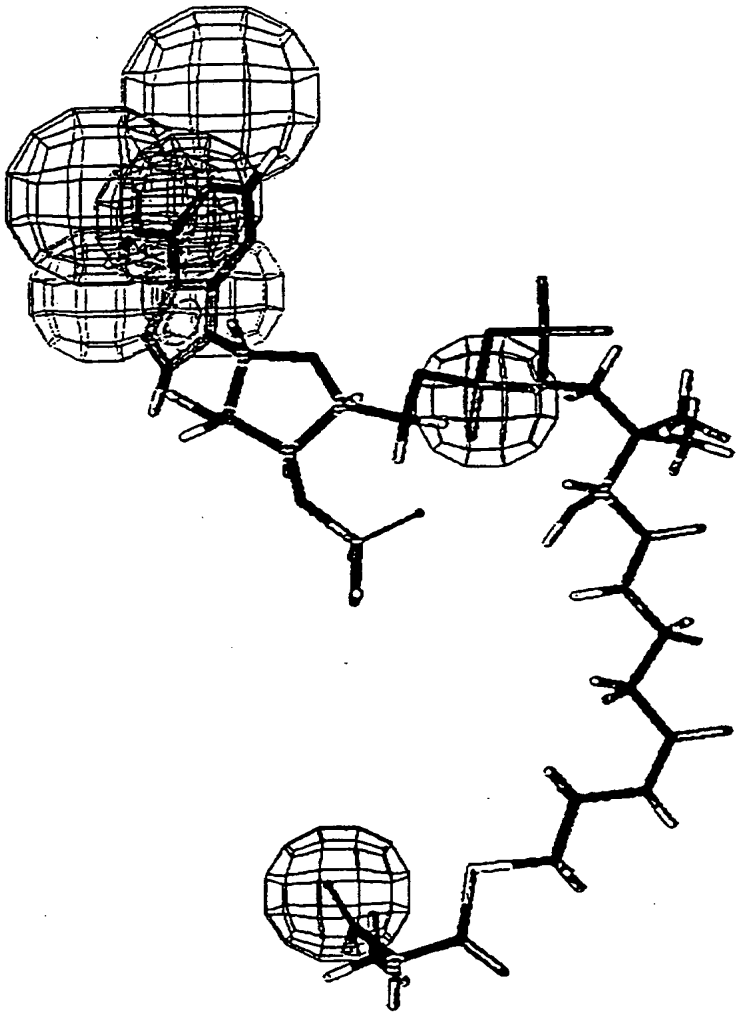


Figure 32

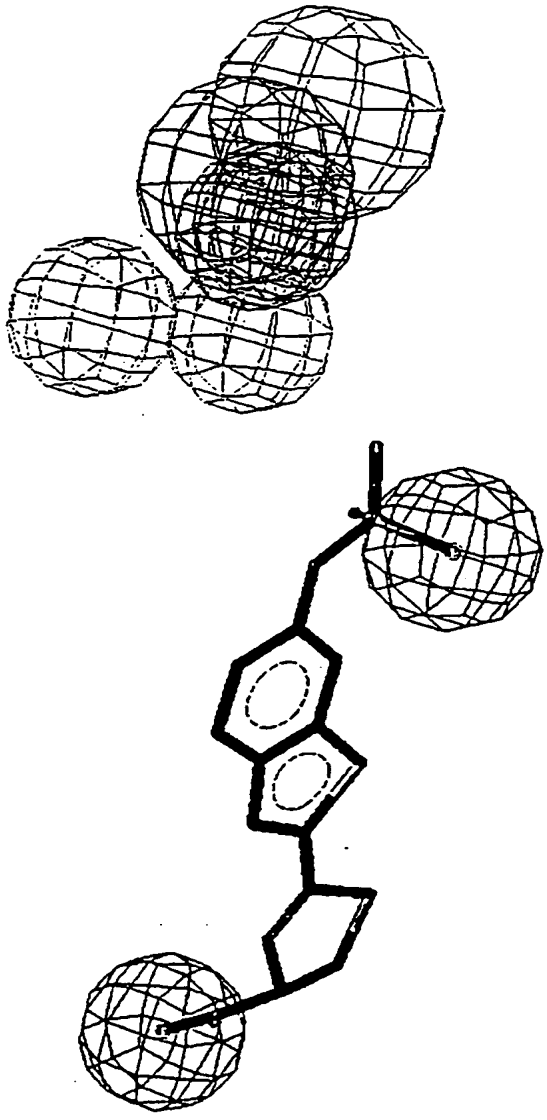


Figure 33

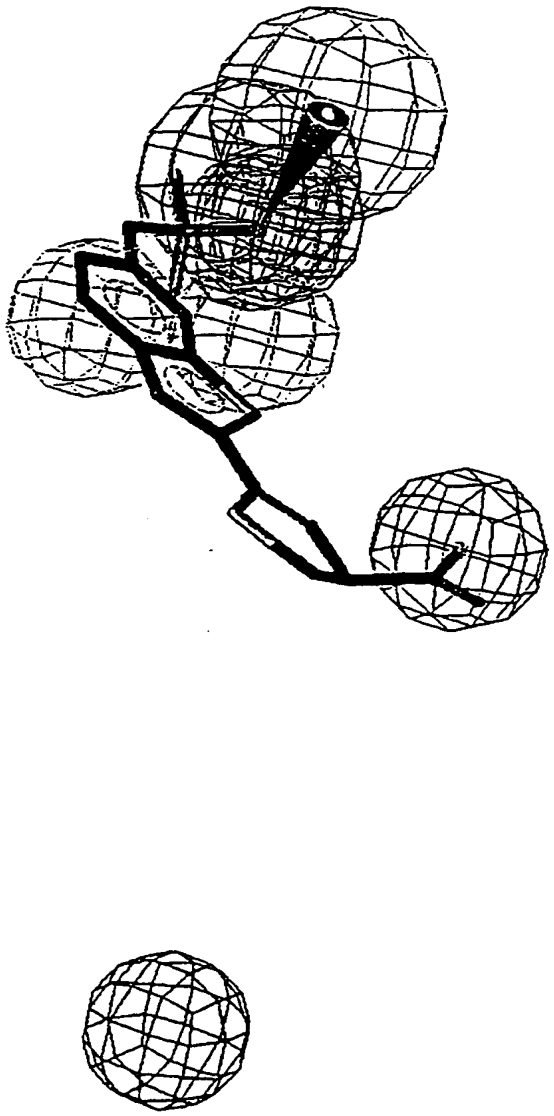


Figure 34

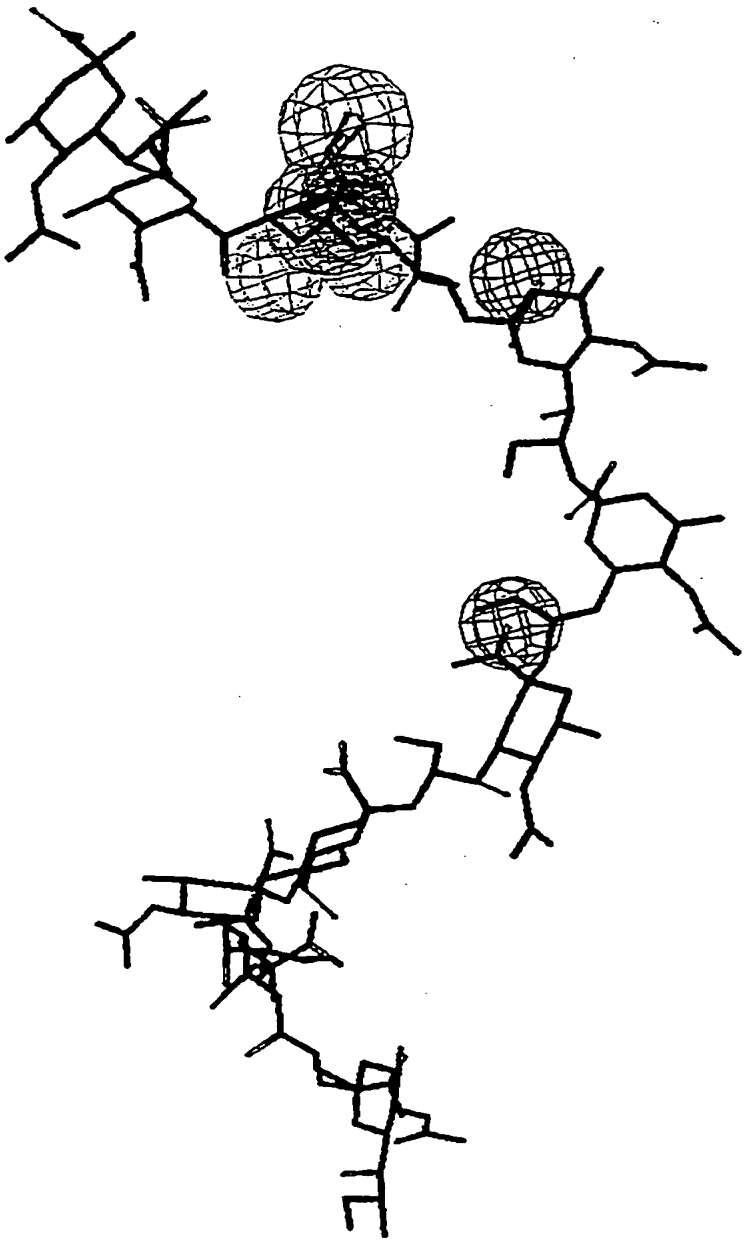


Figure 35

Substitution Net of Peptide GDN...

	A	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	comments
E	-2	2	$	-3	-1	-3	-3	-1	#	-3	-1	#	-3	#	#	-1	-3	-2	-2	D>E>T>others
S	2																-2			
Y	2	#	#	#	-1	-1	-2	-2	-2	-1	#	#	-2	-3	-2	-2	#	#	$	A>Y>G/H/M etc.
A																				
C																				
V	-2	#	0	0	0	0	0	-2	0	-3	-1	-2	-3	#	#	#	$	#	#	V/E/F/G/H/I/L - (D,R,S,T,W,Y not tolerated)
D	0	$	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	D only, no substitutions tolerated
T																				
P	-2	-2	-2	-2	#	#	-2	-3	#	-2	-2	$	-2	#	0	-2	-2	#	-1	P/S, all other substitutions suboptimal or not tolerated
C																				
S																				

Figure 36

List of Compounds Fitting the Combinational Pharmacophore

	CAS Number
Azosulfamide Disodium Salt	000133-60-8
Methylmalonyl Coenzyme A Tetralithium Salt Hexahydrate	104809-02-01
Beta-Nicotinamide Adenine Dinucleotide Phosphate Na-Salt	104809-28-1
Nicotinamide 1,N6-Ethenoadenine Dinucleotide Phosphate Sodium Salt	104809-28-1
Kayarus Supra Violet 5bl Conc	
Nicotinamide Hypoxanthine Dinucleotide Phosphate Disodium Salt	006739-64-6
P1,P5-Di(Adenosine-5') Pentaphosphate Sodium Salt	004097-04-5
Acid Violet 7	004321-69-1
P(1),P(5)-Di(Adenosine-5') Pentaphosphate Pentasodium Salt	4097-04-5
S-Methylmalonyl-Coenzym A Tetralithium Salt	104809-02-01
Acid Violet 5	010130-48-0

Training Set for the Combinational pharmacophore

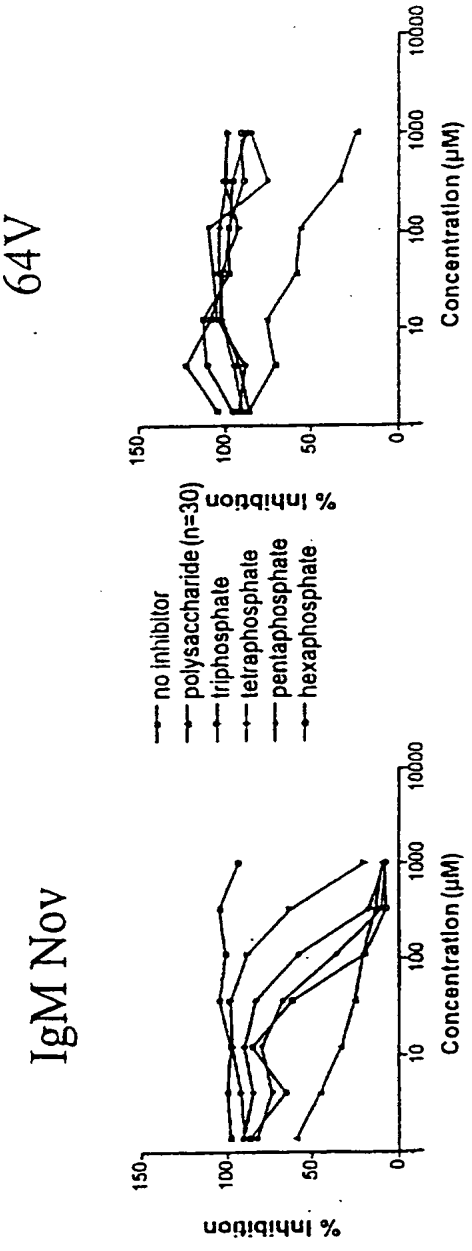
Tripeptide Sequence	Relative affinity
TYR-THR-GLU	1
TYR-SER-GLU	3.3
ASP-ASN-TYR	100
TYR-THR-MET	200
TYR-THR-ASN	200
TYR-THR-GLN	200
TYR-GLY-GLY	1000
GLU-GLY-TYR	2000
ALA-GLY-TYR	3333
TYR-GLY-THR	10000
GLN-GLY-HIS	100000
PRO-GLY-HIS	200000
ASN-GLY-HIS	333333
SER-GLY-HIS	500000
HIS-GLY-PRO	1000000
HIS-GLY-ASN	1000000
HIS-GLY-SER	1000000
HIS-GLY-GLN	1000000
TYR-GLY-ALA	1000000

Figure 37

Replacement Nel of Peplde TIP...

	A	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	comments
D	-1	$	1			0		#			#	#			-1		1	0	-2	D/E/V/H/W>A/S>Y
D	#	$	#			#		#			#	#			#		#	#	#	D only tolerated
E	-1	-1	$			#		-2			#	#	2		#		#	#	#	P>A/D/E>K, (H,N,S,V,W,Y - not tolerated)
_	#	#	#			#		#			#	#			#		#	#	#	I only tolerated
E	#	#	$			#		#			#	#			#		#	#	#	E only tolerated
V	#	#	#			#		#			#	#			#		$	#	#	V only tolerated
M	0	#	#			#		#			#	#			#		#	#	#	M/A
_	0	0	-1			#		#			#	#			-2		-1	0	1	Y>A/I/D/M>V/E>S (H,K,N,P - not tolerated)
Y	0	#	#			#		#			#	#			-2		0	#	$	A/V/Y>S (D,E,H,K,N,P,W - not tolerated)

Figure 38



Inhibition of binding of human anti-polysaccharide antibodies IgM Nov and 64V to solid phase polysaccharide by solution phase haptenic antigens of the diadenosine polyphosphate homologous series.

Figure 39

Binding of IgM Nov to
Ala-scanned mutants of GDN...

GDN FES YAC VDT PCS

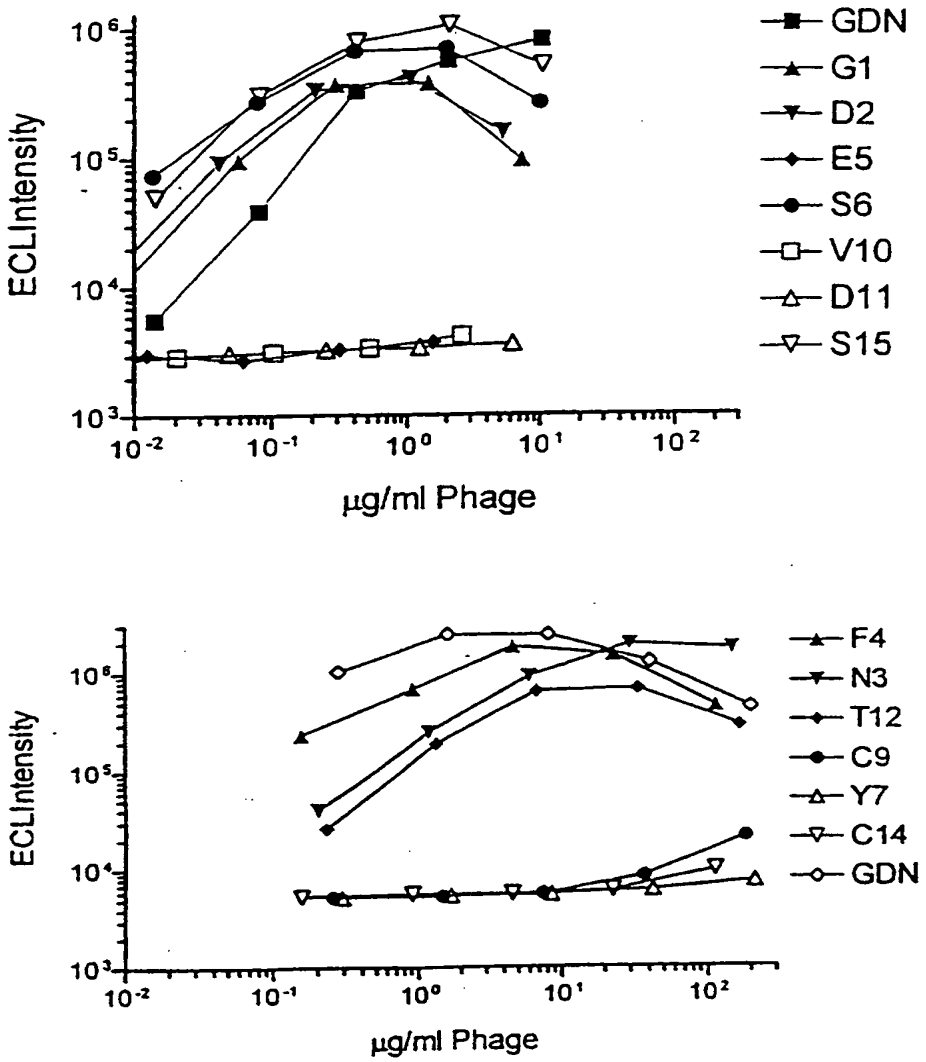


Figure 40

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 97/01518

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/22 C07K7/08 C07K7/06 C07K16/12 A61K38/04
A61K39/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLINICAL MICROBIOLOGY REVIEWS, vol. 7, no. 4, October 1994, pages 559-575, XP002039373 DIAZ ROMERO E.A.: "Current status of meningococcal group B vaccine candidates: capsular or non-capsular" cited in the application	1,53-55, 60-62, 65,67, 68,70, 71,73-75
A	see the whole document	2-52, 56-59, 63,64, 66,69,72

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 September 1997	19.09.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Groenendijk, M

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 98, no. 9, 28 February 1983 Columbus, Ohio, US; abstract no. 65800, MEDVEDEV E.A.: "Effects of neuropeptides, modulators of memory, on background and induced electrocortical activity in rabbit cerebral hemispheres" XP002039375 see abstract & NEIROFIZIOLOGIYA, vol. 14, no. 6, 1982, pages 578-584, ---	1-8,11, 12,15, 18,19, 21,23, 26-31, 33,75
X	CHEMICAL ABSTRACTS, vol. 122, no. 13, 27 March 1995 Columbus, Ohio, US; abstract no. 161287, AGUILAR E.A.: "Molecular definition of the retention parameters of peptides separated by RP-HPLC" XP002039412 see abstract & PEPT.RES., vol. 7, no. 4, 1994, pages 207-217, ---	1-20, 23-27, 30,31, 33,75
X	CHEMICAL ABSTRACTS, vol. 119, no. 23, 6 December 1993 Columbus, Ohio, US; abstract no. 244157, CHO E.A.: "Substrate specificities of catalytic fragments of protein tyrosine phosphatases..." XP002039413 see abstract & PROT.SCI, vol. 2, no. 6, 1993, pages 977-984, ---	1-12, 14-21, 23-27, 30,31, 33,56,75
X	WO 90 06696 A (PRAXIS BIOLOG INC ;RIJKINSINSTITUUT VOOR VOLKSGEZ (NL)) 28 June 1990 The whole document; see esp. Table 8,seq 5 see claims 1-58 --- -/--	1-7,39, 46-52, 56,59, 61-63, 65,66, 68,69, 71,72, 74,75

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter ..al Application No
PCT/GB 97/01518

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 26365 A (UNITED BIOMEDICAL INC ;WANG CHANG YI (US)) 5 October 1995 SEQ ID No. 26	1-9,11, 13-16, 18-20, 23,24, 26,30, 33,40, 42, 46-48,75
X	WO 92 18150 A (ANERGEN INC) 29 October 1992 see page 21, line 36; claims 1-30	1-8, 15-19, 21,40, 46-48, 56,75
X	WO 96 00238 A (PEPTIDE THERAPEUTICS LTD ;LEWIN IAN VICTOR (GB); BUNGY ALI GHOLAM) 4 January 1996 see the whole document	1-8, 15-19, 21,40, 46-48, 56,75
X	GB 2 282 380 A (MERCK & CO INC) 5 April 1995 See esp. SEQ ID NO. 10	41, 46-51, 56,75
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 92, April 1995, WASHINGTON US, pages 4021-4025, XP002039374 WESTERINK E.A.: "Peptide mimicry of the meningococcal group C capsular polysaccharide" see the whole document	1-75

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INTERNATIONAL SEARCH REPORT

national application No.
PCT/GB 97/01518

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Please see Further Information sheet enclosed (obscurities).

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claims 63-68 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

Obscurities :

The scope of the claims 1-21,23-31,33-43,46-75 is unclear and speculative. The claims 1-7 lack any clear indication concerning the structural features and size of the compounds/peptides. The claims 8-21 and 23-31 and 33 define formulas that only consist of variables or contain only one defined amino acid residue of the complete sequence. Moreover expressions like "analogue and homologue derivatives thereof by virtue of one or more amino acid addition, deletion, substitution" (claims 34-36) cannot be considered to be clear and concise definitions of patentable subject-matter (Art.6 PCT).

A complete search for peptides containing a trimeric motif as defined in the claims 39-41 would result in several thousands of documents and can therefore not be considered to be meaningful.

Therefore a meaningful and economically feasible search could not encompass the complete subject-matter of the claims. Consequently the search has only been complete as far as related to sufficiently defined compounds and also to the actually synthesised compounds and (closely) related analogs, that is the compounds encompassed by the claims 22,32,44 and 45. (Art.17(2)(a)(ii) PCT).

Incomplete Search :
Claims searched completely: 22, 32, 44, 45
Claims searched incompletely: 1-21, 23-31, 33-43, 46-75

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inten .nal Application No
PCT/GB 97/01518

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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